QTL Mapping for Discovery and Characterization of an Inhibitor of *Fhb1* in Hexaploid Wheat (*Triticum aestivum*)

A Dissertation
SUBMITTED TO THE FACULTY OF
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

James A. Anderson

May 2017

Acknowledgements

I would like to first and foremost thank my graduate advisor, Dr. James Anderson. You have been instrumental in making me a better all-around researcher and writer. I had expertise in the art of plant breeding, but it was your guidance that taught me the science behind it. You always supported me and believed in my potential, and I thank you so much for that.

My graduate committee also deserves recognition for making me a better scientist. Dr. Gary Muehlbauer taught me so much through great scientific conversations and showed strong interest in my work. Dr. Rex Bernardo helped me better understand quantitative genetics and its application to practical plant breeding. Dr. Ruth Dill-Macky was instrumental in advancing my knowledge of scab and providing the inoculum necessary for this project. Thanks also to Dr. James Kolmer for developing my understanding of plant/pathogen interactions during the early days of my graduate career.

Thank you as well to Xin Li and the Muehlbauer lab for genotyping the *Fhb1*-population. Without your vital contribution, I could have never made the conclusions presented in this study.

Members of the Small Grains staff were critical to the success of this project. Discussions with all of you were some of the most entertaining and helpful conversations of my graduate career. Gary Linkert, Susan Reynolds, Catherine Springer, Roger Caspers, and Galen Thompson managed field plots, offered extensive help with all field operations, and maintained my seed over many years. Jen Flor, Emily Conley, and Karen Beaubien were instrumental in teaching laboratory techniques to a student with little lab experience, and thanks to Emily for fulfilling marker requests in the ensuing years from a remote graduate student. Thank you as well to Dr. Shiaoman Chao, Mary Osenga, and Dawn Feltus at the USDA genotyping lab in Fargo who let me conduct my genome-wide marker work in their lab and helped me enjoy my visits to North Dakota.

I also owe a huge debt of gratitude to my fellow graduate students and undergraduate research assistants. Jen Gee and Matt Nelson provided immense help in the lab, and I enjoyed the conversations we had. Margaret Krause was an incredible worker with great enthusiasm to help with any task. She was an amazing friend and I considered her a protégé, though in this case, I believe the mentee has become the expert. The Anderson lab brought me great friendships and collaborations with my fellow wheat students, Kathryn Turner, Prabin Bajgain, Godwin Macharia, and Ed Quirin. Thank you to Christopher Schaefer, Michael Kantar, Rob Proulx, Landon Ries, Wade Kent, Ilene Jones, Jon Massman, Liana Nice, Addie Thompson, and Eric Koeritz for helping me both scientifically and personally. In particular, Eric, Addie, Jon, Liana, and Kathryn were the best friends I could ask for and you gave me my most memorable graduate school moments. Thanks as well to my oldest and closest friends, Tim Lyon and Tony Hooper, for providing me a friendly ear and periodic breaks from scientific research.

Lastly, I want to extend a huge thank you to my parents, Gene and Barb Seda, and my brother Dan, sister in-law Katie, and nephews Jack and George. It's because of your love and support that I could pursue my intellectual dreams. I am what I am because of you.

Abstract

Fusarium head blight (FHB) is one of the most destructive diseases of wheat worldwide, resulting in decreased grain yield and seed quality, and infected grain that is unacceptable for end use due to the presence of mycotoxins, such as deoxynivalenol (DON). Asian sources of resistance have had the most impact worldwide, with the most prevalent resistance gene in many wheat breeding programs being *Fhb1*, originally mapped in the Chinese variety 'Sumai 3.' DON is the primary *Fusarium graminearum* virulence factor, and *Fhb1* has been successful at preventing epidemics due to its dual role in fungal defense and DON detoxification, resulting in both reduction in DON concentration and prevention of disease spread within the grain head. However, during candidate gene investigation, segregating susceptibility occurred in the homozygous presence of *Fhb1* in the moderately susceptible variety 'Bobwhite.' Other studies have also found resistance conferred by susceptible parents, which along with these candidate gene results, indicate there may be inhibitory genes present in some backgrounds that suppress the effect of FHB resistance genes.

This study was conducted to first identify additional regions of the genome responsible for FHB resistance, and then determine if any of these regions could be inhibiting *Fhb1*. The 260-2 (Sumai 3/Stoa//MN97448 resistant NIL)/Bobwhite population used in the candidate gene study was recreated with separate sub-populations selected for the homozygous presence or absence of *Fhb1*, and genotyped with the 9K Infinium SNP chip. Quantitative trait loci for resistance and correlated traits (FHB spread, FHB

severity, FHB incidence, DON accumulation, visually scabby kernels, 30 head weight, micro test weight, plant height, and heading date) were mapped in each sub-population, and phenotypic analysis indicated polygenic inheritance for all traits. Both populations identified genomic regions coincident with previously reported major genes (*Fhb2* and *Ppd-D1*), as well as a potentially novel QTL on the long arm of chromosome 2A.

The *Fhb2* and 2A QTL regions were highly significant for FHB resistance and exhibited similar additive effects under both *Fhb1* states. The combination of all trials conducted here indicates no interaction between any QTL and *Fhb1*. Although there is no evidence of resistance gene suppression, the results present a thorough investigation of additive gene action for Fusarium head blight resistance in the context of *Fhb1*-mediated resistance. Selection for QTL at multiple loci will enable wheat breeders to develop improved Fusarium head blight resistance, especially in the presence of *Fhb1*.

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Chapter 1

Literature Review

Wheat and the Wheat Genome

Ranking first with over 735 million metric tons produced on over 224 million hectares worldwide in 2015, wheat is the most agriculturally-important food crop. Wheat contributes twenty percent of the total calories consumed by all humans (FAOSTAT 2012). The first wheat arose in the Fertile Crescent region of the Middle East between the years of approximately 6000-7600 BC, and has been classified as a "founder crop" for modern agriculture along with two-row hulled barley, lentils, bitter vetch, and peas (Stallknecht *et al.* 1996). According to Gill *et al.* (2004), wheat was the first crop to be domesticated and is the most recent agricultural crop to have undergone polyploidization. Among the staple food crops of wheat, maize, and rice, wheat is best adapted to growth in temperate regions.

Wheat is also a host to a wide suite of pathogens, most being fungal in origin. Some of the most prevalent and damaging of these diseases are stem rust (*Puccinia graminis* Eriks & E. Henn), leaf rust (*Puccinia triticina* Eriks), stripe rust (*Puccinia striiformis* Westend), *Fusarium* head blight (*Fusarium graminearum* Schwabe), crown rot (*Fusarium pseudograminearum* O'Donnell & Aoki), tan spot (*Pyrenophora tritici-repentis* (Died.) Drechs), Septoria leaf blotch (*Septoria tritici* Rob. ex Desm), and powdery mildew (*Blumeria graminis* f. sp. *tritici* Marchal). This is in addition to Bacterial Leaf Streak (*Xanthomonas translucens* pv. *undulosa* (Smith, Jones and Reddy) Vauterin, Hoste, Kersters and Swings) and Barley Yellow Dwarf Virus (spread through various aphid vectors), which are economically important non-fungal pathogens (Bockus *et al.* 2010).

Common, or bread, wheat (*Triticum aestivum*), like all other polyploid wheat species, is an allo-polyploid that exhibits disomic inheritance. Common wheat represents the final entity resulting from a series of two hybridization events that gave bread wheat its three genomes and allo-hexaploid status (Faris *et al.* 2002). The diploid donor of the A genome is wild einkorn wheat (*Triticum urartu*), and the cultivated einkorn wheat that was among the first crops to be domesticated is *Triticum monococcum* (genome designation AA). This diploid ancestor of common wheat diverged from wheat's wild ancestors in the *Aegilops* genus around 3 million years ago (Gill *et al.* 2004). Approximately 0.8 million years ago, *Triticum urartu* hybridized with the B genome donor that has been postulated to be a close relative of *Aegilops speltoides* (genome designation BB), and allo-tetraploid emmer wheat was formed (Huang *et al.* 2002, Marcussen *et al.* 2014). This wild emmer wheat was *Triticum turgidum* ssp. *dicoccoides*, and the cultivated form was *Triticum turgidum* ssp. *turgidum* (genome designation AABB), which is cultivated durum wheat. The final hybridization event occurred approximately 8,000 years ago (Huang *et al.* 2002) when *Triticum turgidum* hybridized with the wild D genome donor, *Aegilops tauschii* (genome designation DD). This final event produced the allo-hexaploid bread wheat that is the predominant cultivated wheat species, *Triticum aestivum* (genome designation AABBDD).

The reason the bread wheat genome is able to maintain its allohexaploid constitution is due to the *Ph1* locus (Okamoto 1957, Riley *et al.* 1958), located on the long arm of chromosome 5B, that suppresses homoeologous chromosome pairing. This one gene prevents the interpairing of chromosomes from the A, B, and D genomes of bread wheat,

and also keeps wheat chromosomes from pairing with chromosomes of distantly related species and genera consisting of genomes other than A, B, or D. Discovery of the *Ph1* locus and identification of mutants lacking it effectively allowed wheat researchers to transfer alien genetic material into common wheat by enabling chromosome pairing with the alien species (Gustafson and Sears 1993).

The major advantages of hexaploid wheat include larger seeds and much broader adaptability to differing photoperiod and vernalization requirements, differing soil conditions and climates, differing pest problems, and the ability to make a wider variety of food products (Dubcovsky and Dvorak 2007). However, the major consequence of this recent domestication event that led to hexaploid bread wheat and tetraploid durum wheat was the coinciding genetic bottleneck. It's been estimated that the transition to cultivated durum wheat resulted in an 84% loss in diversity, and the domestication of bread wheat led to a 69% diversity loss (Haudry *et al*. 2007). While this bottleneck is not uncommon during any domestication, it does result in a major loss of genetic diversity that could be utilized for future crop improvement.

With the extremely limited use of einkorn and emmer wheats today, the bulk of wheat utilization in the world falls on tetraploid durum wheat and five market classes of hexaploid common wheat that are differentiated by growth habit, kernel hardness, kernel color, and protein content. Due to differences in these characteristics, very specific market classes have arisen. These inherent characteristics cause each of these classes to produce very specific types of consumable products.

Durum wheat is the lone tetraploid wheat currently widely cultivated for human consumption. It is characterized by a spring growth habit, amber kernel color, high protein content (~12-16%), and an extremely hard kernel. In the United States, durum wheat is primarily grown in the Pacific-Northwest, California, and North Dakota. It is milled to produce semolina flour, which is used for pasta production (Beuerlein 2001).

Among the market classes of bread wheat, hard red winter wheat is the most widely grown class in the United States and is characterized by a winter growth habit, hard kernel, red grain color, and medium-high protein content (~10-13%). Approximately 40% of the wheat grown in the U.S. is hard red winter. The major growing region is from the Mississippi River west to the Rocky Mountains, and from southern Texas to South Dakota. This class of wheat is milled and used to make various breads and all-purpose flour (California Wheat Commission 2016).

Hard red spring wheat is characterized by a spring growth habit, hard kernel, red grain color, and very high protein content (~13-16.5%). This class of wheat comes in behind only hard red winter wheat in terms of production in the United States. Its primary growing region is the upper-Midwest states of Minnesota, the Dakotas, and Montana. Due in large part to its high protein content, it possesses superior milling and baking characteristics compared to the other wheat classes and is used almost exclusively to make yeast breads (California Wheat Commission 2016).

Soft red winter wheat is characterized by a winter growth habit, soft kernel texture, red grain color, and low protein content (~8-10%). In the United States, soft red winter

wheat is grown almost exclusively east of the Mississippi River. Due to its low protein content, it's a poor bread-making wheat, and is thus used primarily in the production of cookies and crackers (Beuerlein 2001).

Soft white wheat is characterized by a spring or winter growth habit, soft kernel texture, white grain color, and low protein content (~8-10%). This class of wheat is grown in the Pacific-Northwest and California and is primarily used to produce cakes, pastries, and flatbreads (Beuerlein 2001).

Hard white wheat is characterized by a spring or winter growth habit, hard kernels, white grain color, and high protein content (~12-14%). It is grown predominantly in the Pacific-Northwest and, as the newest class of wheat, is grown on a very small acreage. Due to this, its current market is primarily domestic, but exports are expected to increase as it competes with Australia for the Japanese hard white wheat market. Hard white wheat is used for making yeast and flat breads and oriental noodles (California Wheat Commission 2016).

The hexaploid wheat genome is the largest and one of the most complex crop genomes. A draft genome sequence was created using the cultivar 'Chinese Spring' as the reference genome (International Wheat Genome Sequencing Consortium 2014). More than 75,000 genes were positioned on chromosomes and 106,000 total protein-coding genes were estimated. The genome is approximately 17,000 Mb in size, which is approximately 8-fold larger than maize and 40-fold larger than the rice genome (Gill *et al*. 2004). Over many mapping studies, the total genetic map length has been estimated to be between

2,500 to 3,500 cM (Somers *et al.* 2004, Torada *et al.* 2006). In addition to the sheer size of the wheat genome, it is also very complex, being composed of >80% repetitive DNA (Gupta *et al.* 2008), all of which complicates genome analysis. The overall mega-base-pair:centimorgan ratio is approximately 4.4, which also indicates the genome's complexity and explains the difficulty in cloning wheat genes (Huang 2003).

Principles of QTL Mapping

The concept of mapping genes has been around since the early 1900's. At that time, only recombination frequencies based upon phenotypic observations of simply inherited Mendelian traits could be used to determine the linkage between a locus and trait. Eventually this was improved through the use of cytogenetic stocks that could allow a researcher to effectively identify a locus controlling a trait on a specific chromosome or even chromosome arm. This all changed in the early 1980's with the discovery and subsequent usage of DNA markers. With the dense array of sequence-based markers now available to researchers working on nearly any organism, breeders and geneticists can explore more complex quantitative traits that don't follow simple Mendelian inheritance (Lander & Schork 1994). This technology has also allowed scientists to positionally-clone important genes through fine mapping and candidate gene identification (Huang 2003).

The first step in mapping is population creation. Examples of population types are collections of inbred cultivars, F_2's, backcrosses, recombinant inbred lines, and doubled haploids. A cultivar collection is useful in association mapping studies, where measures

of relatedness are based on linkage disequilibrium between distantly related individuals (Breseghello and Sorrells 2006, Rostoks *et al*. 2006). An F_2 population provides an opportunity to quickly investigate QTL in a Mendelian population, but cannot be completely replicated. Backcrosses are ideal for mapping major genes when the source of resistance is an unadapted line that can be crossed into an adapted genotype (Tanksley and Nelson 1996). This is also the process used to generate near-isogenic lines for fine mapping. Recombinant inbred line populations generate inbred lines from a biparental cross, via single seed descent, that can be highly replicated and offer more allelic combinations than backcrossing. Doubled haploid populations require the ability to generate doubled haploids, but result in completely homozygous genotypes. Regardless of population type, the conventional FHB mapping methodology has been to cross a highly resistant genotype to a susceptible one to identify genomic regions responsible for the large phenotypic difference. This has been highly effective at mapping major-effect loci, but inconsistent in its ability to identify minor-effect QTL (Buerstmayr *et al*. 2009).

The next step is collecting phenotype data on all individuals for the traits of interest, preferably with replication. Members of the population are then genotyped with high quality molecular markers. Examples of DNA-based markers are restriction-fragment-length polymorphisms (RFLP), amplified-fragment-length polymorphisms (AFLP), simple-sequence-repeats (SSR), and single-nucleotide-polymorphisms (SNP). The phenotypic and genotypic datasets are then combined and analyzed to associate specific marker alleles with phenotypic variants (Kearsey and Farquhar 1998).

While methods like single-marker analysis and single-interval mapping are effective in cases of simply inherited traits, the primary method used for complex traits and large populations has been composite-interval mapping as described by Zeng (1994), which combines interval mapping with multiple regression to better estimate marker effects. As with all statistical methods, careful consideration must be taken to determine how many individuals to include in the study, and how many markers to analyze to gain sufficient statistical power while keeping costs low. Too few markers leads to insufficient map coverage, which makes it difficult to identify a selectable marker linked to the QTL of interest. Too few individuals can lead to an underestimation in the number of QTL controlling the trait of interest and an overestimation of the magnitude of effect of those QTL identified (Beavis 1998).

While mapping for the purpose of gene discovery is important, the real utility of QTL mapping comes in its application to plant breeding and crop improvement. Through identification of diagnostic markers for the regions, or even genes, of interest, breeders can utilize marker-assisted selection to improve the genetic base of the lines selected and released from their breeding program. This is done by simply selecting for the marker allele of interest, rather than selecting on the trait alone, the measurement of which is more subject to error, expense, and environmental variance (Anderson 2007). These markers can also be used to stack multiple genes together and determine the combination that will best predict a superior-performing genotype. All of this leads to varieties that will be more resistant to biotic and abiotic stresses, higher yielding, and of greater nutritional or processing quality (Collard *et al.* 2005, Varshney *et al.* 2006).

***Fusarium* Head Blight:**

Fusarium head blight (FHB) was first reported in North America in the 1890's in the eastern region of the United States (Shaner *et al.* 2003). It was considered to be a major threat to wheat and barley production throughout the early part of the 20th century (Stack 1999), and caused infrequent epidemics from 1920 until the 1990's in North America. However, in the 1990's, yearly epidemics occurred throughout the eastern parts of the United States and the Great Plains region. Of largest impact was the major epidemic seen in the hard red spring region of the upper Midwest in 1993 and 1994. This epidemic resulted in an estimated $1 billion loss in 1993 alone, which accounts for one of the largest single season crop losses ever due to a single disease in North America (McMullen *et al.* 1997). The repeated severe epidemics of the 1990's represent the most severe plant disease epidemics since the stem rust epidemics of the 1950's (Windels 2000). Epidemics over the last 20 years have also been observed throughout Asia, Europe, and South America (Shaner *et al.* 2003). Symptoms of the disease are reduced yield, grain weight, seed quality and seedling vigor, discolored and shriveled kernels, and DON accumulation (Windels 2000). All of these lead to a major reduction in market value for the grain produced. The impact felt by these disease epidemics prompted the funding of major research initiatives aimed at identifying FHB resistance and management practices that could reduce the impact of the disease on wheat producers (McMullen *et al.* 2012).

While there are many species of *Fusarium* that can cause FHB in wheat and barley, the predominant ones are *Fusarium avenaceum*, *Fusarium culmorum*, and *Fusarium*

graminearum (Wiese 1987). All species have regional prevalence, with *Fusarium culmorum* favoring cooler climates and being the dominant causal pathogen in infections of northwest Europe, and *Fusarium graminearum* preferring hotter climates and thus being dominant in North American and eastern European infections (Parry *et al.* 1995). Overall, *Fusarium graminearum* is the species most associated with FHB, and this review will focus solely on it, due to its status as the predominant species in North America.

Fusarium graminearum is capable of causing head blight or 'scab' in wheat, barley, rice, and oats and *Gibberella* stalk and ear rot in maize. The teleomorph is a homothallic ascomycete fungus that possesses both idiomorphs of the mating type genes in the same nucleus (Goswami and Kistler 2004). Possessing both idiomorphs allows for both self-fertility and outcrossing, though outcrossing is rarely observed in nature. These mating type genes present in nearly all *Fusarium* species are subject to strong purifying selection (Goswami and Kistler 2004). Research on the biology and genetic structure of *Fusarium graminearum* has been greatly aided by the generation of a whole genome sequence and subsequent genetic and genomic studies on the fungus. This work indicated a genome size of 36.1 Mb across 4 chromosomes, and estimated nearly 12,000 genes and the identification of over 10,000 SNPs (Cuomo *et al.* 2007).

Fusarium graminearum is a primarily monocyclic pathogen that relies very heavily upon environmental factors to initiate infection and subsequent epidemics (Bushnell *et al.* 2003). Initial infection is heavily influenced by temperature, rainfall, dew point, plant growth stage, amount and type of inoculum, and plant morphological characteristics. Unlike many other small grain diseases, the host is only susceptible to FHB infection

during the approximately 20 days following spike emergence, so conditions must be appropriate during that window in order for infections and epidemics to occur.

***Fusarium graminearum* Lifecycle:**

The first essential component for infection is the type and amount of inoculum present in the field. While inoculum can exist on its own in soil, on seed, on weeds, and on previously infected plants, the primary source of inoculum is that living saprophytically on residue from the previous year's crop (Sutton 1982, Bushnell *et al.* 2003, Champeil *et al.* 2004, Goswami and Kistler 2004). When inoculum is present in soil, it usually exists as a saprophyte living on organic matter in the soil (Sutton 1982). Inoculum can exist on seeds, but naturally infected seed isn't likely responsible for initiating FHB epidemics (Shaner *et al.* 2003). However, seed from any host grass can be infected in the laboratory and then the colonized seed spread in the field to initiate large-scale infection in controlled experiments (Dill-Macky and Jones 2000). Mycelium on already infected plants can generate conidia that can pass from plant-to-plant, but the 20-day host susceptibility window is too short to allow multiple infection cycles (Shaner *et al.* 2003). *Fusarium graminearum*'s ability to overwinter on plant debris as saprophytic mycelia means that crop debris is the primary source of inoculum for new outbreaks (Dill-Macky and Jones 2000, Shaner *et al.* 2003, Champeil *et al.* 2004, Goswami and Kistler 2004). The amount of inoculum present to initiate infection is directly proportional to the amount of graminaceous plant residue left in the field following harvest. The fungus survives longest on dead plant tissues that take the longest to degrade, such as stem nodes

(Shaner *et al*. 2003). Fields with residue left from the previous year's corn, wheat, or barley crop contain the most inoculum available to infect the next season's crop.

During the life cycle of *Fusarium* pathogens, four different forms of propagules can potentially cause infection in the host plant. The first is hyphal fragments, and while these have caused infection in inoculation experiments, they have never been implicated in nature as a propagule responsible for causing head blight in cereal crops (Dill-Macky *et al*. 2003). The second minor propagule that can cause infections are chlamydospores. These are survival structures derived from asexual fungal hyphae or conidia. Like hyphal fragments, they have been shown to be capable of infection, but in nature, rarely infect grain heads (Dill-Macky *et al*. 2003). The other two spore types that are responsible for infection are macroconidia and ascospores, which combine to cause nearly all FHB infections.

Macroconida are asexual spores that arise either from individual conidiophores or from groups of conidiophores called sporodochia (Wiese 1987). Hyphae and asexual fruiting bodies develop on plant debris in the field. Macroconidia usually contain 3 to 7 septa and measure 20-105 µm in length by 2-56 µm in width (Champeil *et al*. 2004). The macroconidia have a sickle or canoe-like shape. Macroconidia are usually only produced when temperatures are between 16 °C and 36 °C and there is persistent moisture on the residue in the field (Sutton 1982). This type of inoculum can be present and available to cause infection during the entire crop cycle (Champeil *et al*. 2004).

The primary type of inoculum in natural infections are ascospores. These are sexual spores that are produced in sexual fruiting bodies known as perithecia that develop on plant debris. Formation of the perithecium requires temperatures from approximately 15 °C to 31 °C, low intensity UV light, and persistent moist conditions (Sutton 1982). In addition to these external factors, the mycotoxin zearalenone serves as a regulator in the production of perithecia (Sutton 1982). The perithecia can mature in 9-10 days, but normally require about 2 weeks to mature (Sutton 1982). Environmental factors needed for ascospore production are very similar to those required for perithecia development. Ascospores generally consist of 3 cells and measure about 17.5-26.0 µm long by 3.5-5.0 µm wide (Champeil *et al.* 2004). Unlike macroconidia, mature ascospores are not ejected from the perithecia until the time of head emergence on the host plant (Champeil *et al.* 2004).

The key to infection following propagule development is dispersal of the inoculum. Mature ascospores are forcibly ejected from the perithecia when the temperature is between 13 °C and 22 °C and humidity is 95-100%, and dispersal usually occurs during the nighttime hours (Champeil *et al.* 2004). The effect of rainfall on spore release, however, remains unclear. Spores aren't often released immediately after large amounts of rainfall, but they also aren't frequently released when no free water is present. Dispersal from the perthecia can extend up to 10 mm (Schmale *et al.* 2005), and while transport often occurs within a field, wind currents can deposit the ascospores in excess of 1 km (Francl *et al.* 1999). While aerial turbulence is required for spore dispersal, winds as low as 2-3 m s^{-1} can keep them airborne until they're deposited on a new host

(Prussin *et al*. 2015). The spores can be transported through rain-splash, but this won't carry them farther than a few meters, and transport by insect vectors can occur, but isn't efficient for large-scale dispersal (Shaner *et al*. 2003).

The dispersal of ascospores is in stark contrast to the dispersal mechanism utilized by macroconidia. These asexual spores can be spread via wind currents, though their primary means of transport is through rain-splashing. This may be due to the sticky and hydrophilic properties of the structures themselves, but there is no proof of this (Sutton 1982). In order for rainfall-mediated spread to occur, the macroconidia have to be splash dispersed onto the leaves or stems, and then further onto the grain head (Champeil *et al*. 2004). The limitation of this means of dispersal is that it's limited to within-field transport, meaning macroconidia are not generally responsible for initiating epidemics (Sutton 1982).

After the fungal propagules have come into contact with the host, the next major step in the disease cycle is infection of the host plant. Once an ascospore has been deposited on the wheat spikelet, it will germinate within 3 to 6 hours if the temperature is from 20 °C to 32 °C and there is free water present (Goswami and Kistler 2004). Conidia will usually germinate 5 to 6 hours after deposition, as long as the same environmental conditions are met. These spores may require additional nutrients be present in order for germination to occur, but only at very high spore densities (Bushnell *et al*. 2003). For germination to occur at a maximal rate, high water pressure needs to be achieved with continued wetness for 48 to 60 hours after spore deposition (Sutton 1982). Following germination, the fungus generally produces enough hyphae to grow a mycelium, with

maximal growth being achieved between 12 °C and 28 °C (Bushnell *et al*. 2003). Barley represents the other small grain crop most affected by the pathogen, and while the *Fusarium graminearum* transcriptomes are very similar in infected wheat and barley spikes, host-specific gene expression is observed (Lysøe *et al*. 2011, Harris *et al*. 2016). The most notable differences were in genes associated with transport and secondary metabolism and indicate the ability of the pathogen to adapt to multiple hosts (Harris *et al*. 2016).

In addition to the aforementioned environmental conditions needed for germination of the fungus, the location of deposition is also critical for disease progression. Spores landing on the soft parts of the palea or lemma that consist of a thin walled epidermis or parenchyma cells can easily invade and colonize the flower. Any tissue such as the glumes that contains many chlorenchyma cells can also be easily colonized. After deposition on these tissues, penetration of the epidermal layer occurs through either subcuticular growth or formation of penetration pegs that penetrate the tissue and degrade the cell wall through the use of enzymes such as cellulase, xylanase, or pectinase (Bushnell *et al*. 2003). Direct penetration occurs via the formation of infection cushions, lobate appresoria, foot structures, and infection hyphae (Boenisch and Schafer 2011). Even when the germination occurs on thicker external tissues, mycelia can still find susceptible points to enter and colonize the tissue through subcuticular growth. These alternative points of entry could be wounded cells, gaps between the palea and the lemma, stomates, or the anthers (Bai and Shaner 2004, Goswami and Kistler 2004). Stomates provide a natural and readily available break in the cuticle that provides easy

access to the underlying epidermal layer, and they contain ample amounts of chlorenchyma, which aids in colonization. Whether penetrating through entry of open stomates or colonization of those stomates, the fungus has access to the underlying parenchyma cells (Bushnell *et al*. 2003) once it has breached the host's epidermis.

The pathogen's access directly into the floret between the palea and the lemma is exceptionally difficult. Prior to anthesis the two parts of the flower overlap and don't allow any foreign body to easily enter. Subcutaneous entry across the tissue can occur, but unhindered entry into the flower isn't easily accomplished. However, during dehiscence, the flower is open for four to six days, during which spores can enter the flower from aerial transport, rain-splash, or small insect vectors like mites (Bushnell *et al*. 2003). Anthers are also easily infected and colonized, as are individual pollen grains (Ribichich *et al*. 2000). The anthers themselves have especially high concentrations of choline and betaine, which are known to stimulate fungal growth (Strange *et al*. 1974).

While wheat is resistant to FHB infection prior to flowering due to closed flowers and an unexposed spike, it becomes very susceptible once it reaches anthesis. Although true susceptibility is determined by the genetics of the cultivar, the flower is physically susceptible to attack and colonization up to about 20 days post-anthesis (Dill-Macky and Jones 2000). Likely reasons for the susceptibility of the plant during and after anthesis are the opening of the flower as the kernel develops, the presence of senescing anthers, and a possible decrease in defense reaction efficacy due to increased amounts of metabolites being allocated to grain development rather than pathogen defense (Bushnell

et al. 2003). Due to the short window in which the plant is susceptible (~10-20 days), FHB is relegated to its status as a monocyclic disease (Bai and Shaner 2004).

Following initial infection, the next component of the disease cycle is the spread of the fungus and development of disease symptoms. After the fungus has entered the flower, it first undergoes a brief biotrophic period in which it feeds on living host tissue, and once dead tissue has been generated, it quickly initiates its true necrotrophic relationship with the plant that allows it to spread and colonize the entire head (Bushnell *et al*. 2003). Provided there are no restrictions due to temperature or available moisture, the fungus vigorously colonizes the head after it enters its natural necrotrophic state (Goswami and Kistler 2004). Initial infection of one floret does not prevent infection of other spikelets within the same head (Bai and Shaner 2004). Once the initial infection has occurred, migration to other florets within that spikelet occurs through the vascular bundles of the rachilla (Bushnell *et al*. 2003) and migration to other spikelets occurs through the vascular bundles of the rachis (Goswami and Kistler 2004). There is no evidence of preferential movement of the fungus in any one direction from the initially infected spikelet. While spread of the fungus through the rachis is the primary means of spread, the fungus is also capable of spreading between spikelets over the exterior of the flower during extended wet conditions (Goswami and Kistler 2004). It has been suggested that the clogging of and changes to the vasculature may lead to the premature death of spikelets above the initial point of infection. This is due not to the presence of the fungus and subsequent infection, but rather to the inability of water and nutrients to reach these spikelets (Bai and Shaner 2004).

If the fungus doesn't infect the floret within a few days of anthesis, it can still infect the developing kernel. Approximately 10 days after pollination, the kernel has developed layers of protection against invasion by the pathogen, including the testa, the aleurone, and the pericarp (Bushnell *et al.* 2003). Due to the rigid composition of these layers and the compounds they generate, the fungus has difficulty directly penetrating those layers. However, even in the presence of these protective layers, the kernel can still be invaded. This infection can occur through the micropyle opening and the chalazal tract, or the kernel and endosperm surfaces can become colonized, which ultimately results in the characteristic "tombstone kernel" appearance (Bushnell *et al.* 2003).

Mycotoxin Production:

All head blight causing species produce mycotoxins, and these toxins are dangerous for human and animal digestive systems. The primary mycotoxins that can be produced by *Fusarium graminearum* during an infection are termed trichothecenes and include nivalenol (NIV), deoxynivalenol (DON, or vomitoxin), and the acetyldeoxynivalenol isomers 3-ADON and 15-ADON (Goswami and Kistler 2004). A novel type A trichothecene named NX-2, and its deacetylated form named NX-3, were recently discovered in *F. graminearum* isolates collected from northern Minnesota (Liang *et al.* 2014, Varga *et al.* 2015). The three classified genotypes of *F. graminearum* are nivalenol only producers, DON+3-ADON producers, and DON+15-ADON producers. *Fusarium* strains in North America are dominated primarily by DON+15-ADON producers, but the prevalence of DON+3-ADON producers has increased (Goswami and Kistler 2004). NX-2 producing strains will need to be monitored to determine if they will expand in a

similar manner to the 3-ADON producing strains (Liang *et al.* 2014). In all chemotypes, concentrations of DON are always higher than the acetylated isomers.

All the trichothecenes are terpene-derived molecules that act as fungal virulence factors and are generated by the DON biosynthetic pathway, which is encoded by a 12 gene cluster and three additional linked genes. The *Tri5* gene initiates the first step in trichothecene formation, and is thus the most critical gene in the pathway (Desjardins 2006, Proctor *et al.* 2009). Its expression is especially high at the infection front and downregulated in fully senesced and colonized tissues (Hallen-Adams *et al.* 2011). Production of the NX-2 trichothecene in NX-2 producing strains appears to be the result of a variant in the *Tri1* gene (Varga *et al.* 2015). In addition to aiding in infection, DON has been shown to reduce grain weight and starch and protein content (Snijders 1990, Bushnell *et al.* 2003, Bai and Shaner 2004). Mutant strains lacking the ability to produce DON can infect but are less aggressive than wild-type strains. Jonkers *et al.* (2012) found the Wor1-like protein Fgp1 was needed for pathogenicity and trichothecene production in *F. graminearum.* DON production does occur within infection structures, but is not necessary for the initial biotrophic interaction or for inducing necrosis (Boenisch and Schafer 2011). When DON is present in seedlings, it causes suppression of coleoptile and root growth, and the reduction in grain weight it causes in adult plants implicates DON directly in yield losses (Bushnell *et al.* 2003, Bai and Shaner 2004). DON is water-soluble and can be actively transported through the phloem of the plant, which allows DON to be found in florets that may not actually be infected by the fungus itself (Agyris *et al.* 2003, Bushnell *et al.* 2003).

The pathogen strain, plant part infected, period of colonization, temperature, moisture, competing organisms, time of harvest, and underlying resistance of the cultivar all help explain the differential levels of DON accumulation in wheat (Sutton 1982, Agyris *et al.* 2003, Bushnell *et al.* 2003, Bai and Shaner 2004). The rachis, for example, presents a major barrier to the spread of the fungus, and the pathogen must induce DON biosynthesis to overcome this barrier (Jansen e*t al.* 2005, Maier *et al.* 2006, Ilgen *et al.* 2009). This leads to the rachis accumulating high concentrations of DON, while the kernel and peduncle are relatively less contaminated when all other factors are equal (Bushnell *et al.* 2003). Earlier infections and longer periods of moisture after inoculation cause higher DON content than later infections with drier conditions (Shaner *et al.* 2003). The presence of several amines (Gardiner *et al.* 2009), low pH conditions (Merhej *et al.* 2010), reactive oxygen species (Ponts *et al.* 2006), and phenolic acids (Ponts *et al.* 2011) all act as inducers of the trichothecene biosynthesis pathway (Kazan and Manners. 2011).

Evidence suggests that any relationship between DON accumulation and disease severity is very inconsistent (Argyris *et al.* 2003, Bushnell *et al.* 2003, Champeil *et al.* 2004). Causes could be environmental factors that may promote one and not the other, the strain that's producing the toxin, or the detoxification/conversion of DON to less toxic forms by the host plant. Given this lack of correlation, there will always be some DON present in any infected field, regardless of the resistance displayed by the host genotype since most selection in breeding programs has been for disease severity rather than mycotoxin concentration. DON accumulation can still be especially high in very lightly infected seeds that aren't completely colonized by mycelium (Hallen-Adams *et al.* 2011). Due to

the inherent health concerns of the mycotoxins, the presence of DON is a major concern for the food industry (Bai and Shaner 2004, Champeil *et al*. 2004). Since the epidemics of the 1990's, the acceptable threshold level of DON in finished wheat flour products has been set at 1 ppm by the FDA (Shaner *et al*. 2003). Crops exceeding this threshold are to be rejected by processors, and thus cause major economic losses for producers.

Disease Symptoms and Pathogen Signs:

The first symptoms of head blight are necrotic lesions present on the exterior of the infected floret. These lesions can occur along the entire length of the grain head, but they usually first appear on the center of the head, since these are the first florets with mature anthers. The color of these lesions varies from brown, to dark purple, to black (Goswami and Kistler 2004). If moisture levels in the field are extremely high, the glume and rachis can exhibit a water-soaked appearance. With disease progression, the rachilla and rachis will exhibit the same brown coloration as the infected spikelet due to spread of the fungus. Once the disease has spread across most of the head, the peduncle, just below the head, will also develop the same brown to purple color seen on the rachis (Goswami and Kistler 2004). With time, the entire head becomes bleached and tan in color, the grain within the florets atrophies, and awns become twisted and curve downward (Wiese 1987, Goswami and Kistler 2004).

The signs of the fungus take longer to become visible, but are still clearly identified when infections are severe. Fungal masses (sporodochia) are salmon-orange to pink in color, and colonization results in pink anthers and exterior floral structures. Tissues possessing

this orange to pink color are indicative of extensive mycelial growth (Goswami and Kistler 2004). With time, it may even be possible to observe perithecia on the exterior of the spike (Bushnell *et al*. 2003).

Symptoms and signs are also present on the seed produced by the infected head. Early on, dark brown spots on the seed indicate infection of the kernel, and these can coalesce and cover the entire seed (Wiese 1987). The atrophied seed likely results from the premature maturation of the seed caused by a reduction in the ability of the seed to acquire the nutrients and water necessary for development (Bushnell *et al*. 2003). Signs of the fungus on the kernel appear as a chalky pink to white mycelium covering the kernel and giving the characteristic "tombstone" appearance (Bushnell *et al*. 2003).

Disease and Pathogen Control:

In all wheat growing regions in which FHB is a potential problem, there are several ways in which *Fusarium* head blight can be controlled. The first is through cultural practices involving tillage and crop rotations. Since the major source of *Fusarium* inoculum is from crop residue, the best way to control the disease is through limitation of the presence of infected crop residue on the soil surface. One way to do this is through the use of tillage practices that bury residue below the soil surface or at least minimize the surface exposure. While no-till and conservation tillage practices prevent soil erosion, they also lead to much worse FHB infections due to the residue remaining on the soil surface (Dill-Macky and Jones 2000). Another way to keep inoculum levels low is through the use of crop rotations. This is typically achieved by following a small grains

crop with soybeans or another legume that isn't a primary host for *Fusarium* (Dill-Macky and Jones 2000). The pathogen can colonize dead tissue of many hosts, but it is generally unable to infect legumes during their growth, which minimizes inoculum availability for the next small grains crop.

Fungicides represent a second option for FHB control. While research into fungicide usage uncovered nothing to aid in control of FHB through the mid 1990's (McMullen *et al.* 1997), much has been uncovered since then, and now there are fungicides that consistently aid in decreasing FHB severity and DON accumulation (McMullen *et al.* 2012). Demethylation inhibitor fungicides inhibit sterol production, which is essential for fungal cell wall development (Hewitt 1998). The triazoles prothioconazole, metconazole, and a mixture of prothioconazole with tebuconazole (all demethylation inhibitor type fungicides) are reported to provide up to a 50-60% reduction in damage associated with FHB and DON accumulation (Bradley and McMullen 2008). Some of the best products have been Prosaro (prothioconazole plus tebuconazole, Bayer CropScience) and Caramba (metconazole, BASF) (McMullen *et al.* 2012). However, regardless of effectiveness, there are difficulties with reliance on fungicides. First, most of these applications employ aerial application, which increases expense to the grower. Second is getting adequate and consistent coverage on all heads and avoiding application to wet fields, which hinders efficacy of the fungicide (McMullen *et al.* 2012). Lastly, and most importantly, is the difficulty in deciding to apply and the timing of the application. Given the short window of time in which flowering and infection can occur, fungicide applications must be done once the risk of infection exceeds the grower's threshold, and

applications must occur when all plants in the field are flowering, but before those flowering early have become severely infected (McMullen *et al*. 2012).

A third option for FHB control that's presently under investigation is the use of agents for biological control. Prospects for nutrient competition from *Cryptococcus*, induction of localized resistance from *Lysobacter*, and production of antifungal metabolites by *Bacillus* have been studied, as have other possible biological control agents (McMullen *et al*. 2012). These agents have been shown to provide protection when used in combination with demethylation inhibitor fungicides, but there is no evidence that biological control alone can decrease FHB severity or mycotoxin accumulation (Jochum *et al*. 2008, Yuen *et al*. 2010, Halley *et al*. 2010), though they may be able to provide late season control after fungicide application is no longer possible.

While all of these methods of control can be effective, the most important and effective method of controlling the disease is through development and deployment of resistant cultivars. While fungicides and biological controls are inherently reactionary and cultural practices can only reduce inoculum availability, the use of resistant cultivars provides a base level of resistance to infection over the course of the entire season. Though generation of these resistant lines is critical and less expensive for the farmer than chemical applications, in many years these lines may still need to be coupled with a fungicide application to gain economic control of FHB.

Forms of FHB Resistance:

Unlike many other small grains diseases (i.e. rust diseases), there is no evidence of race-specific genetic resistance to *Fusarium* head blight. Resistance to this disease can come in morphological or physiological forms, all of which are characterized as non-race specific, quantitative forms of disease resistance.

The first of these resistance types is known as passive, or morphological, resistance and is characterized by disease avoidance and not active genetic resistance. Key morphological traits that contribute to this passive resistance are heading date, flower opening/anther emergence, spike density/compactness, plant height, and presence/absence of awns (Mesterhazy 1995, Parry *et al*. 1995). Later heading plants tend to be more resistant to infection than earlier heading varieties. This is likely due to the plant entering its susceptible stage of development long after fungal spores have been released. This is especially the case if this delay coincides with inadequate environmental temperature and moisture at the time of flowering (Parry *et al*. 1995). The effect of flower opening and anther extrusion has been debated for years (Takegami 1957, Liang *et al*. 1981), but it's understood that the presence of dead anthers aids infection. If anthers are retained inside the floret, this facilitates susceptibility, as do florets that open and stay open. The effect of spike structure is of lesser magnitude, but still well known. Varieties with high spikelet density and compact heads are more susceptible than heads with more widely spaced spikelets due to the proximity of infected spikelets to uninfected spikelets and the microclimate caused by the spikelet density (Mesterhazy 1989). Heads that remain erect are also more susceptible than those that "nod," due to moisture retention (Mesterhazy

1989). Plant height is also a key feature, as it's well-known that taller plants are more resistant than shorter varieties, because spores must travel farther from the soil surface to come into contact with the spike and cause infection (Mesterhazy 1989). It's also been observed that awned wheat varieties are more susceptible than awnless varieties (Mesterhazy 1989). This is likely due to increased humidity between awns and greater moisture retention within the floret (Snijders *et al.* 1994).

While these passive forms of host resistance aid in control, many of them have negative correlations with desirable agronomic traits. Due in part to this, breeding for FHB resistance focuses on improving the host for active (physiological) forms of resistance. The first form of active resistance (known as Type I resistance) is defined as resistance to the incidence of infection, meaning the plant resists the initial spikelet infection (Schroeder and Christensen 1963, Mesterhazy 1995, Bai and Shaner 2004, Buerstmayr *et al.* 2009). The second form (known as Type II resistance) is defined as the resistance to fungal spread, meaning the plant gets infected but doesn't allow the fungus to spread and colonize the remainder of the spike (Schroeder and Christensen 1963, Mesterhazy 1995, Bai and Shaner 2004, Buerstmayr *et al.* 2009). Also recognized is resistance to accumulation of trichothecenes, meaning the plant may be susceptible to FHB, but yet does not accumulate high concentrations of mycotoxin or detoxifies much of what it does accumulate (Miller *et al.* 1985, Snijders and Perkowski 1990, Mesterhazy 1995, Mesterhazy *et al.* 1999, Bai and Shaner 2004). Resistance to kernel infection, meaning the seed itself doesn't get colonized by fungus, regardless of its presence in the floret

(Mesterhazy 1995, Mesterhazy *et al.* 1999, Bai and Shaner 2004), has also been described by multiple researchers.

Screening for Active Resistance:

With environmental factors heavily impacting FHB development, it is critical that any researcher studying the genetics of resistance utilize experimental designs that minimize this environmental impact and allow for discrimination of genotypes. Given the multiple forms of resistance present and other confounding morphological factors, investigators must also screen for all of these traits in order to truly determine the best genotypes (Fuentes *et al.* 2005). These methods of screening can employ field experiments, greenhouse experiments, and/or laboratory procedures.

Type I resistance screening is done via artificial inoculation and subsequent counts of the number of infected plants in a selected sample (Dill-Macky *et al.* 2003, Bai and Shaner 2004, Buerstmayr *et al.* 2009). Fewer total spikes will exhibit infection if the line is resistant to initial infection. Screening should be performed under a relatively low inoculum load, so the entire head isn't overwhelmed by the fungus, since even resistant genotypes will eventually become infected (Bai and Shaner 2004).

Unlike resistance to initial infection, Type II resistance assessment can be done in multiple ways and is more complex. The first method is through field screening under artificial inoculation. The rating is then taken as the percentage of spikelets per head that exhibit symptoms of infection. It is possible to have multiple points of initial infection, but all infected spikelets are considered to have become infected due to spread of the

fungus from the initially infected florets. This trait is essentially a measure of Type II resistance, and is commonly referred to as field or disease severity. The other method of assessing Type II resistance is by point inoculating a single central floret, often in a greenhouse or growth chamber, and then counting how many non-inoculated spikelets become infected (Dill-Macky *et al.* 2003, Bai and Shaner 2004, Buerstmayr *et al.* 2009). This trait is referred to as resistance to spread in the spike or Type II resistance.

Since mycotoxins can still accumulate in varieties that are resistant to FHB in the field, measuring DON concentration in all field inoculated plots is critical. All means used to do this are destructive in nature and result in a determination of the DON concentration in the grain. The techniques employed to measure this concentration in harvested grain are the enzyme-linked immunosorbent assay (ELISA), gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), gas chromatography/electron capture (GC/EC), and thin layer chromatography (TLC) (Mirocha *et al.* 2003).

Assessment of kernel infection can be done in several ways. Kernel infections can be indirectly measured through the weight of a specified number of spikes (30 head weight) or through the weight of a specified volume of grain (test weight) (Dill-Macky *et al.* 2003). Both of these assume that reductions in grain weight are caused by FHB. While this assumption may occasionally be incorrect, it still serves as a suitable proxy for determining yield loss as a result of *Fusarium* infection. The other primary method of assessing kernel infection is through the rating of *Fusarium* damaged kernels (FDK) or visually scabby kernel ratings (VSK). Those methods are subjective and determine the

percentage of grain that exhibits visual signs of the fungus by comparing the grain sample to known standards (Jones and Mirocha 1999).

Experimental design and resource allocation are critical in order to minimize the overwhelming effect of environment, minimize expense, and give the best potential for infection and disease progression. In field screening experiments, where environmental conditions cannot be under complete control, irrigation with a misting system is vital (Dill-Macky *et al*. 2003). A researcher may not be able to manage the temperature, but by providing moisture, the researcher can provide the free water needed to promote *Fusarium* infections. While temperature can be controlled in a greenhouse experiment, moisture is still needed in the greenhouse environment to promote infection. This is achieved by either putting the plants in a dew chamber for a designated period of time or placing plastic bags over the inoculated heads and utilizing the water generated by the plant's own respiration to promote infection (Mesterhazy 1995). As with the assessment of all other quantitative traits, multiple locations and years of screening must be performed and resistant and susceptible checks must be screened to gauge infection. However, even genotypes stable for resistance across years will exhibit large amounts of variation across environments (Groth *et al*. 1999). Research indicates that visually scabby kernel ratings are the most consistent measure of field resistance across years (Fuentes *et al*. 2005).

With the testing design established, the final component in screening for resistance is the choice of inoculum and the method of inoculation. In most cases, macroconidia are used because the spores can be readily produced, don't require time for maturation, can be

easily quantified, and their hydrophilic nature allows them to be delivered in suspension. As a result, they can be either sprayed onto the plots in the field or introduced into the floret in the greenhouse (Dill-Macky *et al*. 2003). Spray inoculation should be done at anthesis, and repeated 3-7 days later so each plot receives two applications. Use of ascospores as an alternative in field trials is challenging, but the ample infection they give can offset some of that difficulty. They need time to mature and can't readily be put into suspension, thus the most effective way to utilize ascospores is to spread *Fusarium* colonized grain (grainspawn) inoculum produced using corn or a small grain (Dill-Macky *et al*. 2003). This method allows for spore dispersal over several weeks and requires less labor, since there's no need to make multiple trips into the field to inoculate by hand over many anthesis dates. The use of grainspawn inoculum reduces the experimental control, since there's no control of spore deposition. For field experiments, it is generally considered best to apply a mix of fungal isolates collected from previous seasons in order to remove variance due to isolate differences across environments (Dill-Macky *et al*. 2003). In a greenhouse screening, inoculating with a single, well-characterized, and aggressive isolate is recommended to promote more reproducible infection levels.

Sources of Resistance and Mapped QTL:

Sources of native resistance to FHB have been identified from South America, Europe, and the United States, but the primary origin of FHB resistance has been Asia. Resistance has been documented in winter, spring, and durum lines, as well as from several alien species. Resistance from Europe and South America have been derived from winter wheat varieties, while resistance from North America and Asia has come

primarily from spring wheat genotypes. Regardless of region, many of the sources of resistance identified are taller and later flowering than their susceptible counterparts. Breaking linkages of resistance with these negatively correlated traits is vital to gaining usable FHB resistance.

The most thoroughly studied sources of resistance are all from the Asian region, which has reported regular epidemics (Bai and Shaner 1994). The primary sources of resistance observed in this region are from the variety 'Sumai 3' and the Chinese landrace 'Wangshuibai,' with smaller contributions from a few other lines, including lines descended from 'Sumai 3.' While these exotic sources of resistance show very good resistance to FHB, they also are very tall and late flowering when grown in North America, traits which have negative agronomic impacts on grain yield. In addition to mapping these resistance genes, work must be done to minimize the size of the introgressed genomic segments and remove the linkage drag associated with them.

The most heavily studied and utilized source of resistance to FHB is from the Chinese variety 'Sumai 3' and 'Sumai 3'-derived lines. All QTL mapped from this source have major effects on resistance to fungal spread and resistance to DON accumulation (Waldron *et al.* 1999, Buerstmayr *et al.* 2009). The most significant FHB resistance reported to date is that conferred by the 'Sumai 3' 3BS QTL. This QTL typically explains 25-30% of the variation in Type II resistance and DON accumulation (Bai *et al.* 1999, Lemmens *et al.* 2005, Pumprey *et al.* 2007). The 3BS QTL shows greater FHB resistance with less yield penalty than the 5A QTL from 'Wangshuibai' (Tamburic-Ilincic 2012). When the semi-dwarfing gene *Rht-D1* is present, both the 'Wangshuibai'

5A and ‘Sumai 3’ 3BS QTL are needed to compensate for the susceptibility conferred by the semi-dwarfing allele at this locus (Lu *et al.* 2011). Fine mapping has been performed on the 3BS region by Cuthbert *et al.* (2006) and Liu *et al.* (2006) and this QTL has officially been designated *Fhb1*.

The other major ‘Sumai 3’ QTL was found on chromosome 6BS. It accounts for slightly less than 10% of the trait variation in most studies, but is reported to be consistent and stable (Waldron *et al.* 1999, Shen *et al.* 2003, Lin *et al.* 2004, and Yang *et al.* 2005a). This QTL was fine mapped and named *Fhb2* by Cuthbert *et al.* (2007). Minor effect QTL for Type II resistance have also been identified on chromosomes 2BL and 2AS in the ‘Sumai 3’ derived line ‘Ning 7840’ (Zhou *et al.* 2002). A QTL in the centromeric region of chromosome 7A that explained 20% of the trait variation for Type II resistance and DON accumulation was discovered in a Chinese Spring-Sumai 3-7A disomic substitution line (Jayatilake *et al.* 2011).

Given the large impact of the *Fhb1* gene, many studies have been undertaken to determine its physiological function in FHB resistance. Gunnaiah *et al.* (2012) used metabolic profiling to propose that the gene is responsible for cell wall thickening. However, the most widely accepted function of the *Fhb1* gene is as a detoxifier of the DON virulence factor. A UDP-glucosyltransferase from *Arabidopsis thaliana* was shown to be responsible for detoxification of DON and 15-ADON (Poppenberger *et al.* 2003). The utility of this gene family in DON detoxification has been shown multiple times (Shin *et al.* 2012, Kugler *et al.* 2013, Schweiger *et al.* 2013b, Li *et al.* 2015). The major finding was made by Lemmens *et al.* (2005) and supported by Steiner *et al.* (2009)

showing in hexaploid wheat that *Fhb1* was responsible for converting DON to DON-3-*O*-glucoside either by direct production of DON-glucosyltransferase or by regulating the production of such an enzyme. Limitation of the virulence factor was proposed for the strong Type II resistance and the resistance to DON accumulation seen in lines expressing *Fhb1*.

Studies have been conducted to investigate sequence differences between *Fhb1* alleles and to positionally-clone the gene. Liu and Anderson (2003b) used synteny between 3BS and rice chromosome 1S to develop improved sequence-tagged site (STS) markers for the 3BS QTL region. Five SNPs based on ESTs in the region accounted for ~50% of phenotypic variation and could be used in marker assisted selection (Bernardo *et al.* 2012). Eight haplotype blocks were identified within a 3.1 Mb region of the short arm of chromosome 3B using 266 wheat accessions, with *Fhb1* located in block 6 (Hao *et al.* 2012). Following fine mapping work (Cuthbert *et al.* 2006, Liu *et al.* 2006), Liu *et al.* (2008) conducted the most thorough attempt to clone *Fhb1* to that point. *Fhb1* was isolated to a 261 kb region consisting of 7 putative genes. Through both conventional and transgenic efforts, resulting segregation for resistance was observed in the homozygotes of each candidate. From this study, a diagnostic marker was discovered and developed (an STS marker designated UMN10) and this marker has been widely used for marker-assisted selection (Liu *et al.* 2008). More recent cloning efforts have been aided by release of the 'Chinese Spring' reference genome (International Wheat Genome Sequencing Consortium 2014). Fine mapping showed repressed recombination across an 860 kb interval spanning *Fhb1* and revealed 28 candidate genes, including 13

with high gene model confidence (Schweiger *et al*. 2016). Transcriptional analysis found that inoculation with the fungus and inoculation with DON detected mostly different sets of differentially expressed genes and that the rachis node is responsible for Type II resistance (Hofstad *et al*. 2016). Finally, positional cloning revealed the presence of a pore-forming toxin-like gene (*PFT*) in the *Fhb1* interval responsible for FHB resistance (Rawat *et al*. 2016). This cloned gene is predicted to encode a chimeric lectin that provides resistance to the fungus, but does not confer DON resistance, which is likely the effect of another gene in the *Fhb1* interval (Rawat *et al.* 2016).

'Wangshuibai' resistance, and that from 'Wangshuibai'-derived lines has been mapped in several studies, and the QTL identified are the second most deployed FHB resistance genes and QTL, behind only 'Sumai 3'-derived sources. The novel QTL identified in this line exhibits a strong association with Type I resistance, but has little impact on Type II resistance. Minor effect QTL were found on chromosomes 2D, 5B, and 7A (Jia *et al*. 2005) and on chromosome 2A (Ma *et al*. 2006). The first major effect QTL identified was a QTL for Type I resistance on chromosome 4BL that explained up to 18% of trait variation (Lin *et al*. 2006). This QTL was further fine mapped and subsequently designated *Fhb4* (Xue *et al*. 2010). The most important QTL region mapped from 'Wangshuibai' has been for Type I resistance on the short arm of chromosome 5A (Lin *et al*. 2006). This one gene accounts for the most widely deployed Type I resistance in all wheat growing regions. Xue *et al*. (2011) fine mapped this region and designated the resulting gene region as *Fhb5*.

Other resistant Asian lines have been investigated, and QTL other than those found in 'Wangshuibai' and 'Sumai 3' have been discovered, all with smaller effects. 'Wuhan 1' contributes a Type II resistance QTL on chromosome 2DL and 'Nyu Bai' contributes a field severity QTL in the centromeric region of chromosome 3B (Somers *et al.* 2003, McCartney *et al.* 2007). The doubled haploid line 'DH181' possesses a QTL for Types I and II resistance and resistance to kernel infection on chromosome 2DS (Yang *et al.* 2005a). The Korean variety 'Chokwang' has significant Type II resistance QTL on chromosomes 4BL and 5DL (Yang *et al.* 2005b). A QTL for Type II resistance was also mapped on the short arm of chromosome 3A in 'Huapei 57-2' (Bourdoncle and Ohm 2003). The first stable QTL reported on chromosome 7D was mapped to the long arm in the Chinese landrace 'Haiyanzhong' (Li *et al.* 2011).

The main source of South American resistance to FHB is from the Brazilian variety 'Frontana' (Schroeder and Christensen 1963). It possesses primarily Type I resistance with some impact on Type II and resistance to kernel infection. All QTL from 'Frontana' have been found using biparental mapping populations. The significant QTL identified from 'Frontana' are on chromosomes 2B (Steiner *et al.* 2004, Szabo-Hever *et al.* 2012), the long arm of 3A (Steiner *et al.* 2004, Mardi *et al.* 2006, Szabo-Hever *et al.* 2012), 5A (Steiner *et al.* 2004, Szabo-Hever *et al.* 2012), 6B (Steiner *et al.* 2004), the short arm of 7A (Mardi *et al.* 2006), and 7B (Szabo-Hever *et al.* 2012). The Type I resistance of 'Frontana' appears to be highly quantitative and no "large-effect" QTL have been identified (Buerstmayr *et al.* 2009). Of those identified, the largest-effect and most

unique QTL is the one discovered on the long arm of chromosome 3A (Yabwalo *et al.* 2011).

Resistance from Europe has primarily focused on the varieties 'Arina,' 'Dream,' 'G16-92,' 'Cansas,' 'F201R,' 'Goldfield,' 'Sincron,' and 'Renan.' QTL for Type II resistance on the 1B/1R translocation chromosome and on chromosome 1D were found in 'Sincron' using protein storage markers (Ittu *et al.* 2000). However, as more useful markers were never developed, these QTL haven't been widely utilized in breeding. Mapping of 'F201R' uncovered QTL for Type II resistance on chromosomes 1B, 3A, and 5A (Shen *et al.* 2003). 'Renan' contributed QTL on chromosomes 2A and 5AL that are not coincident with plant height or flowering time (Gervais *et al.* 2003). A QTL for Type I resistance was found on chromosome 7B in the variety 'Goldfield' and implicated in active resistance to incidence of infection due to its lack of association with flower morphology traits (Gilsinger *et al.* 2005). A QTL for field severity in the variety 'G16-92' was found on the long arm of chromosome 2B (Schmolke *et al.* 2008). 'Dream' exhibited a QTL for field severity, that wasn't associated with plant height or flowering time, on the long arm of chromosome 2B (Schmolke *et al.* 2005). QTL for field severity on chromosomes 1BS, 3DL, and 7BS are present in the moderately resistant variety 'Cansas,' and are free from negative agronomic trait correlations (Klahr *et al.* 2007). The European accession 'PI277012' possesses a novel resistance QTL on chromosome 5AL (Chu *et al.* 2011).

The majority of FHB resistance mapping work performed on European winter wheat has been done on the moderately resistant Swiss variety 'Arina' (Buerstmayr *et al.* 2009).

Independent studies on 'Arina' reported QTL on 4AL and 3BL (Paillard *et al.* 2004), on 1BL and 6BS (Semagn *et al.* 2007), and on 6BL (Draeger *et al.* 2007). In each study the QTL were not associated with agronomic traits. The susceptible parent 'Forno' also conferred resistance QTL on 3AL and 3DS (Paillard *et al.* 2004), as did the susceptible parent 'NK93604' on 1AL, 7AL, and 2AS (Semagn *et al.* 2007). The semi-dwarf gene *RhtD-1* was also mapped in 'Arina' and is associated with field severity (Draeger *et al.* 2007). This was the first study to uncover the relationship between FHB resistance and this height locus, with the susceptible parent, 'Riband,' possessing the semi-dwarfing *RhtD-1b* allele. This allele conferred susceptibility to FHB when present. Given this negative agronomic correlation and the lack of any repeatable QTL found in 'Arina,' none of these loci make clear targets for marker-assisted-selection, regardless of the obvious resistance present (Buerstmayr *et al.* 2009).

Mapping of native resistance in the United States of America has been focused on the moderately resistant winter wheat varieties 'Ernie,' 'Heyne,' and 'Truman.' 'Ernie' is a soft red winter wheat from Missouri that possesses QTL on chromosomes 2B, 3B, 4BL, and 5A, all of which are associated with Type II resistance (Liu *et al.* 2007). 'Heyne' is a hard red winter wheat from Kansas that contributes QTL for Type II resistance on chromosomes 3DS, 4DL, and 4AL (Zhang *et al.* 2012). Recent work on the variety 'Truman' identified several QTL, with the strongest residing on 2DS (McKendry 2012). The findings are preliminary, but this locus is consistent in location and effect and is associated with Type I and Type II resistance, and resistance to DON accumulation.

While there is clear need for improvement in hexaploid bread wheat, the need is even greater in tetraploid (durum) wheat. Very little resistance is known in this germplasm pool, but recent research has been dedicated to mapping possible sources of resistance in this crop. 'Strongfield' possesses a QTL for resistance to FHB spread on chromosome 2BS (Fedak *et al.* 2006). A single highly significant QTL on chromosome 5BL was mapped in the variety 'Lebsock' (Ghavami *et al.* 2011). A significant QTL for Type II resistance has been mapped to chromosome 7AL of *Triticum dicoccoides* (Kumar *et al.* 2007). Resistance was found in several Tunisian lines that carry novel haplotypes from known hexaploid and tetraploid sources, but more work is needed to characterize this material (Huhn *et al.* 2012).

With so few large-effect QTL identified in bread wheat and durum wheat, research has also been conducted to find resistance in several alien species. QTL for field severity in *Triticum macha* (Georgian spelt wheat) are reported on chromosomes 2A, 5A, and 5B (Buerstmayr *et al.* 2011) and for Type I resistance on 4AS (Steed *et al.* 2005). A QTL was mapped in *Thinopyrum ponticum* to the distal end of the long arm of chromosome 7el (Shen *et al.* 2004). More work was done to reduce the size of this chromosomal region for ease of introgression (Shen and Ohm 2007), and the region has since been introduced to bread wheat chromosome 7D and named *Fhb7* (Guo *et al.* 2015). Potential resistance has also been reported in *Elymus humidas, Elymus racemifar, Roegneria kamoji,* and *Leymus racemosus* (Ban 1997, Chen *et al.* 2005, Oliver *et al.* 2005). Qi *et al.* (2008) mapped *L. racemosus* and identified a significant Type II association on the short arm of chromosome 7Lr#1, and designated this gene *Fhb3*. *Fhb6* was identified in

Elymus tsukushiensis and introduced to the short arm of bread wheat chromosome 1A with a 20% improvement in disease severity (Cainong *et al.* 2015).

Resistance Gene Inhibition:

As previously discussed, many resistance QTL have been discovered that have come from well-known resistant germplasm sources. There are many cases of alleles for FHB resistance contributed by susceptible parents as well. Sometimes these are due to passive resistance (associated with flower structure, heading date, or height), but in other cases they are QTL conferring active resistance from the susceptible parent. The initial 'Sumai 3' mapping work identified QTL for resistance on chromosomes 2AL and 4B in the susceptible line 'Stoa' (Waldron *et al.* 1999). The 2AL QTL explained 14% of the phenotypic variation for resistance to fungal spread, nearly equal to the effect of *Fhb1* in the same population. 'Alondra' has a resistance QTL on chromosome 2DS (Shen *et al.* 2003), 'Patterson' has a resistance QTL on 5BL (Bourdoncle and Ohm 2003), and 'AC Foremost' has resistance mapped to 3A (Yang *et al.* 2005b). A 'Frontana' mapping study found resistance QTL on 1B and 2A derived from 'Remus' (Steiner *et al.* 2004), 'Forno' contributed a resistance QTL on 5BL in a population with 'Arina' (Paillard *et al.* 2004), 'Ritmo' possesses resistance QTL on 1DS and 7AL (Klahr *et al.* 2007), and the highly susceptible variety 'Wheaton' has a disease severity QTL on 1AS (Li *et al.* 2011).

These QTL for active resistance are masked in the presence of multiple susceptibility genes in the susceptible genotype, but are observed in structured populations. The first study to investigate this phenomenon was performed by Handa *et al.* (2008). In this

study, a doubled haploid population from the cross of 'Sumai 3' by 'Gamenya' was generated and mapped. The 'Gamenya' allele at the 2DS QTL found in this study conferred Types I and II resistance when present, while the 'Sumai 3' allele conferred susceptibility. Through rice genome synteny, they determined that the causative gene was likely a multidrug resistance-associated protein (MRP) encoding gene, but this conclusion has not been confirmed. A QTL in this region of the genome that conferred resistance to DON accumulation was also found in other 'Sumai 3' derived populations (Yang *et al*. 2005b, Basnet *et al*. 2012). The possible MRP gene appeared to provide Type II resistance through DON detoxification and existed in a gene complex also containing the height gene *Rht8*, which acted pleiotropically on Type I resistance, with the semi-dwarf allele from 'Sumai 3' contributing to susceptibility (Handa *et al*. 2008).

The potential for susceptibility factors and resistance gene suppression became even greater in a pair of studies looking at durum wheat. A resistance QTL on durum chromosome 3A was only found when the chromosome was removed from the remainder of the genome of susceptible variety 'Israel A' (Stack *et al*. 2002). This implies there were other factors in the genome suppressing the resistance coming from 3A. Garvin *et al*. (2009) mapped regions that may be acting epistatically to mask this resistance gene. They found a QTL on chromosome 2A that consistently enhanced susceptibility even when compared to the allele present in the moderately susceptible line 'Langdon.' This is supported by the epistatic effect of QTL on 2A and 5AS that suppress a 6BS resistance QTL (Fedak *et al*. 2006). Findings such as these imply that regions of the genome exist that interact with resistance QTL to inhibit their effects. Where such susceptibility QTL

do exist, it's unknown at this point what their molecular function is. They may produce proteins that directly enhance susceptibility, or they may be producing sRNA's that post-transcriptionally silence the resistance gene product through the mechanism of RNAi (Jones-Rhoades e*t al.* 2006, Llave *et al.* 2002, Mallory and Vaucheret 2006).

Chapter 2

Mapping an Inhibitor of *Fhb1*

Introduction

Fusarium head blight, (FHB), is a major problem in wheat production throughout the world, and particularly in the upper Midwest. FHB is primarily caused by the fungal pathogen *Fusarium graminearum* and results in severe economic losses caused by reductions in seed quality and grain yield, as well as accumulation of mycotoxins such as deoxynivalenol (McMullen *et al.* 1997, Goswami and Kistler 2004). Consumption of these mycotoxins can cause dangerous health issues in humans and animals and led the US Food and Drug Administration to provide advisory levels on the amount of DON allowed in food (Desjardins 2006). Consequently, developing high yielding cultivars with enhanced levels of FHB resistance is a major goal of many wheat breeding programs.

In excess of one hundred FHB resistance QTLs have been mapped across a wide range of genetic mapping studies (Buerstmayr *et al.* 2009), with most of these only contributing minor levels of resistance. The largest effect QTL maps to the short arm of chromosome 3B and has been named *Fhb1* (Waldron *et al.* 1999, Bai *et al.* 1999, Liu and Anderson 2003a). This QTL was derived from the Chinese cultivar 'Sumai 3' and typically explains 25-30% of the phenotypic variation in FHB resistance. *Fhb1* displays resistance to fungal spread and DON accumulation and is the most widely deployed source of FHB resistance. A pore-forming toxin-like gene was recently positionally cloned and shown to be responsible for much of the anti-fungal activity associated with *Fhb1*, though a gene for DON resistance has not been isolated (Rawat *et al.* 2016).

The preponderance of previous FHB studies identifying regions of resistance conferred by the susceptible parent (Waldron *et al.* 1999, Shen *et al.* 2003, Bourdoncle and Ohm 2003, Somers *et al.* 2003, Yang *et al.* 2005, Steiner *et al.* 2004, Paillard *et al.* 2004, Klahr *et al.* 2007, and Li *et al.* 2011) point to the complexity of FHB resistance and contribute to the hypothesis that susceptibility loci may mask resistance genes, or directly inhibit them, in susceptible genotypes. As *Fhb1* represents such a major source of FHB resistance, the possibility of inhibition in some genetic backgrounds following introgression would be detrimental to variety development. Zhu *et al.* (2014) showed that *Fhb1* is ineffective in durum wheat backgrounds, which provides further support for genomic suppression of *Fhb1*. Although not related to *Fhb1*, a FHB susceptibility QTL was found on the long arm of chromosome 2A in the wild emmer wheat genotype 'Israel A,' when introduced into durum wheat, that may suppress tetraploid resistance genes (Garvin *et al.* 2009).

An attempt was made by Liu *et al.* (2008) to positionally clone *Fhb1* by first identifying seven putative genes in a 261 kb region aligning to the position of *Fhb1* in a 'Chinese Spring' chromosome 3B BAC library. 'Sumai 3' cosmid clones of these candidate genes were transformed into the FHB-susceptible cultivar 'Bobwhite' and all resulted in moderately susceptible phenotypes. The resistant near-isogenic line from the fine mapping population of Sumai 3/Stoa//MN97448, '260-2,' was crossed with 'Bobwhite' to create several F_3 families. Susceptibility was again observed in families homozygous for the presence of *Fhb1* (Fig. 1). That effect was observed in greenhouse screens and field studies under artificial inoculation. These results provided sufficient evidence to

hypothesize that the effect of *Fhb1* can be masked in the 'Bobwhite' background. In addition to observing the effect in inbred lines, F_1 plants only displayed partial resistance. This result implies that the presence of even a single susceptibility allele at an inhibitor locus supersedes the expected resistance phenotype conferred by dominance at the *Fhb1* locus, or that no dominance exists at all. These results led to the hypothesis of this study: that there is a resistance gene inhibitor suppressing *Fhb1*, in particular in the 'Bobwhite' genetic background. Inhibition was hypothesized to be simply inherited as the result of a very small number of large effect genes, though polygenic inheritance could be caused by multiple inhibitors acting additively.

The objectives of this study were to map QTL contributing to FHB resistance, and investigate their potential to inhibit *Fhb1*. To do this, we developed a recombinant inbred line population using the resistant NIL, '260-2,' and 'Bobwhite' as parents, and then partitioned sub-populations fixed for the presence or absence of *Fhb1*. This methodology isolated the resistance gene, while restricting loci in the remainder of the genome to a maximum of two alleles, which allowed us to analyze the remainder of the genome and develop conclusions about which genomic regions were associated with *Fhb1* inhibition.

Materials and Methods

Plant Materials:

The FHB resistant hard red spring NIL used for fine mapping *Fhb1*, '260-2' (Pumphrey *et al*. 2007, Liu *et al*. 2008), was crossed to the susceptible spring variety 'Bobwhite'. The resulting population was inbred via single seed descent to the F_5 generation. F_5 lines were grown in the field in St. Paul, MN during the summer of 2010. During this generation, three individuals per line were tissue sampled in the field, DNA was extracted, and all individuals were screened with the UMN10 marker (Liu *et al*. 2008). Individuals homozygous for the presence of the UMN10 resistant allele were selected for the *Fhb1+* population and individuals homozygous for the absence of the UMN10 resistant allele were selected for the *Fhb1-* population. A seed increase generated 124 $F_{5:7}$ RILs in the *Fhb1+* population and 110 $F_{5:7}$ RILs in the *Fhb1-* population. These were used for greenhouse and field screening, with both populations mapped independently. The parents '260-2' and 'Bobwhite', the susceptible NIL '260-4', and the varieties 'Alsen' (moderately resistant) (Frohberg *et al*. 2006), and 'Wheaton' (susceptible) (Busch *et al*. 1984) were included as checks in the field and greenhouse screenings. 'MN00269' (susceptible), 'BacUp' (moderately resistant) (Busch *et al*. 1998), and 'Roblin' (susceptible) were included as checks in the field experiments only.

Inoculum Preparation:

Greenhouse:

Greenhouse inoculations were performed using macroconidia as the infective propagule. The isolate used was designated *Fg4* and characterized as a Midwest 15A-DON producer (Gale *et al*. 2007, Quirin 2010). This isolate was collected in South Dakota by Dr. Xiuling Zhang and has been maintained on soil at 4 °C in the Dill-Macky Laboratory at the University of Minnesota (Isolate #10108023). The inoculum was produced on mung bean agar (MBA) plates. Stored soil culture was transferred to MBA plates and incubated at room temperature (22 °C) for seven days (12 hours of light per day, cool white and blacklight fluorescent). Plates were then flooded with 15 mL of sterile distilled water per plate and the agar surface rubbed with a sterile L-shaped glass rod to dislodge spores (macroconidia). To increase inoculum, the spore suspension was transferred to additional MBA plates at a rate of 1.5 mL per plate, and spread over the surface of the plate using a sterile L-shaped glass rod. Plates were incubated at room temperature (22 °C) for seven days (12 hours of light per day, cool white and blacklight fluorescent). Spores were harvested by spraying the colony surface with approximately 10 mL of sterile water per plate, and the resulting suspension was sieved through two layers of cheesecloth to remove any agar pieces or large pieces of hyphal tissue (R. Dill-Macky, personal communication). Spore concentration was determined with a hemocytometer and the suspension diluted to the target concentration of 100,000 conidia mL^{-1} with sterile water. The solution was divided into 50 mL aliquots and stored at -80 °C until use.

St. Paul Field Trials:

Field inoculations in St. Paul for both 2011 and 2012 were performed using macroconidia as the infective propagule. The 2011 macroconidial suspension consisted of a mixture of 50 isolates collected primarily from northwest Minnesota between 2005 and 2010 from infected wheat, barley, oat, and corn fields. The 2012 mixture consisted of 30 isolates collected from infected wheat and barley fields in northwest Minnesota between 2007 and 2011. Inoculum was prepared by the University of Minnesota Small Grains Pathology Laboratory. Inoculum production and quantification was performed according to Dill-Macky *et al.* (2003). Prepared inoculum was stored at -20 °C at a final concentration of 800,000 condia mL^{-1}.

Crookston Field Trials:

Field inoculations in Crookston in 2012 were performed using grain spawn (colonized corn kernels). Inoculum was prepared according to Gilbert and Woods (2006) by the Northwest Research and Outreach Center in Crookston. Approximately 4.5 kg of maize kernels were soaked in water for 20 hours, drained, autoclaved in stainless steel trays twice over successive days, and then cooled. Each tray of corn kernels were inoculated by mixing 3-5 PDA (potato dextrose agar) plates colonized with a single isolate with 150 mL sterile water and 0.2 g of streptomycin sulfate. The trays were incubated for 14 days under sterile conditions, and then the inoculum was spread out in the greenhouse to dry and stored in burlap bags. Inoculum consisted of 6-10 isolates of *F. graminearum* previously collected from commercial fields in northwest Minnesota.

Phenotyping for FHB Resistance:

Greenhouse:

Data was collected from three greenhouse seasons to evaluate Type II resistance in the *Fhb1+* population. The entire RIL population was screened in the Fall 2010 and Fall 2011 greenhouse seasons, and a subset of the population with inconsistent results were screened again in the Spring 2012 greenhouse. All of the aforementioned phenotypic checks were planted in each season.

12.7 cm square pots were filled with a 1:1 mix of steamed field soil and Scotts Metro-Mix potting media (Scotts-Sierra Horticultural Products, Marysville, OH) that also incorporated 10 mL of 14:14:14 slow release Osmocote fertilizer (Scotts-Sierra Horticultural Products) into each pot. After pots were filled, 5 seeds of a single genotype were planted into each pot at a depth of 1.5 cm. Four replications were planted, resulting in four randomized complete blocks with four total pots per entry. After plants reached the 3-leaf stage, pots were thinned to 4 plants each to allow ample room for growth. Only primary spikes were inoculated, except in cases where there were less than four plants in a pot and where tillers with large spikes, comparable in size to the main spikes, were available to inoculate, thus bringing the total number of spikes inoculated in the pot to four. The inoculation protocol was as described by Cuthbert *et al.* (2006), with modifications. Only spikes that had reached anthesis, as determined by the presence of anthers shedding pollen, were inoculated. All plants at this stage were marked with colored flagging tape, with different colors of tape used to distinguish inoculation dates.

A central spikelet was marked with a non-toxic Sharpie marker (Sanford Corporation, Oak Brook, IL) to designate the spikelet to be inoculated and a single floret was inoculated by introducing 10 µL of conidial suspension into the developing flower using a Hamilton PB600-1 repeating syringe (Hamilton Robotics, Reno, NV), between the palea and lemma. This allowed for colonization of all floral tissues of the inoculated floret, including the stigma and retained anthers. Zippered 7.6 x 15.2 cm Fisherbrand Bitran Specimen Storage Bags (Fisher Scientific, Atlanta, GA) were placed over the inoculated spikes. These plastic bags contained the moisture produced by plant transpiration and they provided the free water necessary for infection. Bags were left sealed over the spike for 48 hours before being removed and discarded. Twenty-one days after inoculation, the percent of available spikelets that were infected, excluding the inoculated spikelet, was recorded and used as a measure to assess Type II resistance.

Field Screening:

Assessment of field resistance was performed in three different environments: 2011 St. Paul, MN; 2012 St. Paul, MN; and 2012 Crookston, MN. Each nursery consisted of both the *Fhb1+* and *Fhb1-* populations and all checks. In the 2011 trial, three complete randomized replications of the $F_{5:7}$ *Fhb1+* population and two randomized replications of the $F_{5:7}$ *Fhb1-* population were grown. In both 2012 environments, two randomized replications of each population were planted. Methods of inoculation and disease assessment were performed according to Fuentes *et al.* (2005).

The 2011 St. Paul nursery was planted on May 7 and the 2012 St. Paul nursery was planted on April 27. In all seasons, 1.5 meter single row plots were planted with 0.3 meters between the rows. The macroconidial inoculum, comprised of a mixture of isolates, was applied using a CO_2-powered backpack sprayer fitted with a TeeJet flat fan nozzle #80015SS (TeeJet Technologies, Wheaton, IL) operated at 40 psi. 1 L bottles containing the inoculum were thawed, mixed with 7 L of water and 10 mL of polysorbate 20 (Tween 20), and then sprayed onto the wheat heads by passing the spray nozzles over each row 3-4 times to achieve a volume of 33 mL m^{-1} of row length at a concentration of 100,000 conidia mL^{-1}. All plots were inoculated twice in this manner. The first inoculation occurred when the plot reached 50% anthesis and the second inoculation occurred 3-4 days later. The entire scab nursery was mist irrigated for 15 minutes immediately following inoculation, and then again for 10 minutes every hour between 5 pm and 8 am for two weeks in order to promote infection. FHB ratings took place 21 days after inoculation in 2011 St. Paul and 19 days after inoculation in 2012 St. Paul. Total spikelet counts were taken on three randomly selected heads to determine an average number of spikelets per spike for the plot and used as the denominator in the calculation of percent infected spikelets. The number of infected spikelets were counted on each of twenty randomly selected heads, avoiding any late tillers. FHB severity was calculated as the average percentage of infected spikelets per head for each plot. FHB incidence was the percentage of the twenty heads evaluated that had one or more infected spikelets. Heading date was recorded as the day the plot reached 50% anthesis. Plant height was recorded in centimeters from the soil level to the tip of the grain head on three

randomly selected plants per plot ignoring obvious outliers. The height for the plot was determined from the mean of the three measurements.

The 2012 Crookston nursery was planted on April 30 and plot dimensions were identical to the St. Paul location. Inoculated corn kernels were spread in the field during jointing, approximately three weeks before anthesis, at the rate of 56 kg ha^{-1}. A second application was spread one week later. The nursery was mist irrigated for ten minutes every hour between 4 pm and 11 am starting at inoculum application and ending at the time of disease rating. FHB rating date was approximately three weeks after anthesis. Rating methods were the same as used in the St. Paul nurseries, although heading date in Crookston was recorded as the day that approximately 50% of spikes had fully emerged.

Post-Harvest Evaluation:

In all field environments, approximately 50-75 heads (including tillers) were collected at harvest ripeness from the plot with a hand sickle and placed into a labeled paper bag. Bags were then dried at approximately 35 °C in a greenhouse for at least two weeks. Thirty fully developed main spikes were selected from each sample, weighed to determine thirty head weight, and threshed. Heads were threshed using an Agriculex Spt1 bulk thresher (Agriculex, Inc., Guelph, Ont., Canada) with the aspiration turned off to avoid discarding the smaller, FHB-damaged seed. Further cleaning to remove chaff was done using a Model SLN4 seed cleaner (Rationel Kornservice A/s, Esbjerg, Denmark) that consisted of sizing screens and a blower used at a low setting to avoid the

loss of FHB-damaged seed. Any remaining awns, glumes, and rachis pieces were removed by hand.

Visually scabby kernel (VSK) ratings were taken on each sample. Approximately 200-300 kernels were assessed visually to determine the percentage of kernels that showed signs of infection (Jones and Mirocha 1999). If major inconsistencies were observed within a line at a location, the samples were scored a second time. Samples were bulked across replications for micro test weight and DON analysis for each RIL in both 2012 locations, but replications of each check were analyzed separately. Micro test weights were performed on bulked samples by pouring cleaned seed into a 15.7 mL copper vessel (20 mm in diameter and 50 mm in height). A ruler was used to level the sample at the top of the vessel and the sample was weighed. Entries without enough grain to fill the micro test weight vessel were counted as missing data. Samples were then ground and submitted for mycotoxin analysis. DON concentration was determined by the Mycotoxin Diagnostic Laboratory in the Department of Plant Pathology at the University of Minnesota. Individual replications were analyzed in 2011 and for all checks in all environments, while the bulked replications were utilized for each RIL for both 2012 locations. Extraction and analysis was performed using gas chromatography/mass spectrometry (GC/MS) as described by Mirocha *et al.* (2003).

DNA Extraction:

During the summer of 2010, leaf tissue was sampled from F_5 plants after jointing stage in the field and used to determine the allele present at the UMN10 marker and to determine

the composition of the two RIL populations. Leaf tissue for mapping analysis was sampled from each RIL comprising the *Fhb1+* population ($F_{5:8}$ generation), and the parents '260-2' and 'Bobwhite', in the Fall 2011 greenhouse when the plants reached the 3-leaf stage. All tissue samples were from the middle of a single leaf blade and measured approximately 4 cm in length. Tissue was placed in microtubes and put on ice. Samples were transferred to -20 °C storage, freeze dried (lyophilized), and returned to -20 °C storage until needed. One stainless steel bead of 0.3 cm diameter (Rio Grande, Albuquerque, NM) was added to each microtube and all tubes were capped. Tissue was pulverized using a TissueLyser (Qiagen Inc., Valencia, CA) for three segments of 2 min each at a frequency of 30 Hz. Extraction was then performed using a BioSprint 96 plant kit (Qiagen Inc.) according to the instructions provided. Final DNA extract was eluted into 200 µL of 0.1X TE buffer and stored at -20 °C until needed. DNA quantification was done using a Synergy 2 SL Luminescence microplate reader (BioTek Instruments, Inc., Winooski, VT). DNA quality was assessed using 1% agarose gel electrophoresis.

Genotyping:

DNA samples for the UMN10 analysis were serially diluted to 40 ng/µl while the mapping population DNA was diluted to 80 ng/µl. All dilutions were made using 0.1X TE buffer. The UMN10 marker was run on DNA extracted from the two RIL populations and the parents using the protocol of Liu *et al*. (2008). After PCR amplification, the amplicons were separated using polyacrylamide gel electrophoresis.

High density genotyping using the 9K Infinium SNP chip from Illumina (Illumina, Inc., San Diego, CA) (Akhunov *et al*. 2011) was performed initially on the *Fhb1+* population and parents, and later on the *Fhb1-* population by Xin Li, at the USDA-ARS Small Grains Genotyping Laboratory in Fargo, ND, under the supervision of Dr. Shiaoman Chao. Allele cluster analysis was performed on the *Fhb1+* population using GenomeStudio (Illumina, Inc.), with '260-2' designated as the B allele and 'Bobwhite' designated as the A allele. Over 1800 polymorphic SNP markers were identified in each population for inclusion in mapping analysis.

Statistical Analysis:

Raw data for each line/trait combination was entered by replication, and outlying datapoints were removed from further analysis if their standard deviation was more than double the mean standard deviation. This removed two 30 head weight and three VSK datapoints from the 2011 dataset. Raw data for field traits was subjected to spatial analysis based on check performance using GenStat software (VSN International, 2012). For each trait, the adjusted data was compared to raw data via standard deviation magnitudes and distributions to determine if the process had removed variability and improved the dataset. As this only led to minimal improvements in the *Fhb1+* population, spatial adjustments were not performed on the *Fhb1-* population data.

Analyses of variance were conducted using JMP (SAS Institute, v.10) to test for genotype-by-environment interactions, with genotypes and environments treated as fixed effects. Significance tests used the genotype-by-environment term to test for genotype

and environment significance and the residual error term to test for genotype-by-environment significance. Broad sense heritabilities were calculated on an entry-mean basis from the analysis of variance using the equation $h^2 = \sigma^2_G/(\sigma^2_G + \sigma^2_{GE}/E + \sigma^2_e/RxE)$ where σ^2_G is the genetic variance, σ^2_{GE} is the genotype-by-environment variance, σ^2_e is the pooled error variance, E is the number of environments, and R is the number of replications.

Genotypic data obtained from the Illumina SNP chip was used to generate genetic linkage maps. Linkage analysis in the *Fhb1+* population was performed using JMP Genomics (SAS Institute, v.10) with a maximum threshold of 0.55 for estimating the recombination fraction between markers, a p = 0.05 cut-off for segregation tests using false discovery rate as the multiple testing method, and a recombination fraction cut-off of 0.40 for map order construction. Markers with greater than 12% missing data were excluded from analysis, as were individual RILs with greater than 15% missing data, leaving 116 RILs and 1650 SNPs available for linkage analysis. Linkage maps were created using the Kosambi map function (Kosambi 1943), and groups were broken when a 35 cM gap existed between two adjacent markers. Linkage mapping in the *Fhb1-* population was performed with JoinMap (Kyazma B.V., v.4.1) by Xin Li (unpublished) using the 102 RILs with less than 12% missing genotypic data. Once marker order was determined, results were compared to those found by Cavanagh *et al.* (2013) for the entire 9K chip, to determine chromosomal identity.

QTL mapping was performed in both populations using Windows QTL Cartographer version 2.5 (Wang e*t al.* 2007) on individual environment traits, as well as across-

environment trait means. Composite interval mapping was performed across all linkage groups using forward and backward regression with a window size of 10.0 cM, walk speed of 1.0 cM, five control markers, and a 10% probability for inclusion and exclusion of any marker in the model. Empirical permutation testing (Churchill and Doerge 1994) was performed using 1,000 permutations per trait at a significance level of $p = 0.05$ using the QTL package in R (Broman *et al.* 2003), resulting in a LOD (logarithm of odds) significance threshold of 3.2 for all traits. Multiple interval mapping with a significance threshold of $p = 0.05$ was performed in Windows QTL Cartographer version 2.5 (Wang *et al.* 2007) to test for epistatic QTL.

After significant QTL were identified in the *Fhb1+* population, significant SNP markers associated with the major QTL on chromosome 2A, as well as the microsatellites GWM644, linked to *Fhb2* (Cuthbert *et al.* 2007), and GWM311 and GWM382, linked to the 'Stoa' QTL (Waldron *et al.* 1999, Anderson *et al.* 2001), were screened in the *Fhb1-* population and the '260-2' and '260-4' NILs and standard checks using DNA from $F_{5:10}$ progeny of the winter 2013 seed increase. Microsatellites were amplified according to their published PCR conditions (Roder *et al.* 1998) and visualized using polyacrylamide gel electrophoresis. Individual SNPs were converted to the Kompetative Allele-Specific PCR (KASP) platform. KASP marker design was performed by LGC Genomics (Hoddesdon, UK) and data was visualized using KlusterCaller version 3.4.1.36 (LGC Genomics). Single marker analysis was employed in QTL Cartographer using these loci and models were run in JMP to test for relevant trait associations in the *Fhb1-* population.

Results

Phenotypic Results

Trait distributions were near normal for all resistance traits except VSK, DON concentration, and Type II greenhouse resistance in the *Fhb1+* population, as determined by the Pearson chi-squared test at a significance level of $p < 0.05$ (Table 3). Greenhouse data especially skewed toward the resistant phenotypes (Fig. 3). The continuous variation observed indicates polygenic control and implies that multiple QTL exist for each resistance trait. Both populations studied had similar distributions, although a shift towards susceptibility was observed in the *Fhb1-* population, relative to the *Fhb1+* population. For all traits evaluated, transgressive segregation was observed in both directions. The susceptible checks '260-4,' 'MN00269,' 'Wheaton,' and 'Roblin' were all more susceptible than 'Bobwhite,' though '260-4' was more resistant than 'Bobwhite,' with respect to greenhouse FHB spread and DON (suppl. Table 1). 'BacUp' was always more resistant than '260-2,' while 'Alsen,' with *Fhb1*, consistently had a similar level of resistance to '260-2' (suppl. Table 1). Significant genotype-by-environment interactions were observed for all traits in both populations and coefficients of variation were between 14% and 55% for all disease resistance traits (Table 3). Broad sense heritabilities were between 0.73 and 0.89 for those same traits (Table 3).

The resistance traits measured were generally well-correlated across both environments and traits (Tables 1 and 2). Increased FHB severity correlated with reduced test weight ($r = -0.46$), increased DON accumulation ($r = 0.48$), and an increased percentage VSK ($r =$

0.58). Plant height was poorly correlated with most resistance measures. Plant height had no effect on DON accumulation (r = 0.00), but taller plants had slightly lower FHB field severity (r = -0.09), and a lower percentage VSK (r = -0.17). Heading date had inconsistent and unexpected correlations to the disease resistance traits. Heading date was negatively correlated with FHB severity in the 2012 Crookston environment (r = -0.21), and positively correlated in St. Paul (r = 0.17). Overall, a later heading date led to greater mycotoxin accumulation (r = 0.27) and a reduced 30 head weight (r = -0.41). The correlation of the 2011 DON levels to all other traits measured was different in magnitude, and in some cases direction, from that observed in 2012. There is a strong and positive correlation between greenhouse resistance and FHB severity (r = 0.60), grain DON concentration (r = 0.42), and VSK (r = 0.36).

Linkage Mapping Results:

Initial cluster analysis, performed on the 9,000 assayed SNPs, identified more than 1,800 polymorphic SNP markers in the *Fhb1+* population. Following removal of markers with excessive segregation distortion or missing data (>12%), 1650 polymorphic SNPs remained. The chromosomal identities of these 1650 markers are indicated in Table 4. There were 685 polymorphic SNPs from the A-genome, 751 from the B-genome, and 110 from the D-genome. Recombination analysis placed these markers into 30 individual linkage groups. Following removal of RILs with greater than 15% missing genotype calls, these groups were joined to form 23 larger and more distinct linkage groups using the remaining 116 RILs. Sixteen of these linkage groups represent whole chromosomes consisting of all the available markers mapped to that chromosome in the preliminary 9K

chip consensus map (Cavanagh *et al*. 2013). The exceptions are chromosomes 2A, 5A, and 6D, which were each split into two smaller linkage groups. Chromosome 3D was the smallest linkage group represented with a total genetic distance of 6.1 cM, while chromosome 7B was the largest at 137.8 cM. No chromosome 4D linkage group could be constructed using the five polymorphic 4D SNPs. The total genetic distance covered by this linkage map was 1,565 cM. Once linkage groups were formed, duplicate markers co-locating to the same genetic position were removed, and the marker with the least missing data was retained in the map. This resulted in 352 total SNPs in the final linkage maps shown in Figure 4 (SNP names provided in suppl. Table 2). The average chromosome length across all groups mapped is 68 cM and the largest gap between any two adjacent markers was 30.4 cM. The average distance between any two adjacent markers was 4.7 cM.

Following removal of SNPs and RILs with excessive missing genomic data, the resulting residual heterozygosity per chromosome was 0-8% by SNP and 0-13% by RIL. Heterozygotes were converted to missing marker genotypes during mapping, which resulted in an increase in missing marker datapoints and duplicate loci. Linkage analysis conducted on the *Fhb1*- population by Xin Li (unpublished), using similar methods, resulted in 782 total polymorphic SNPs and 430 non-redundant marker loci, for an approximate map length of 1,744 cM across 102 RILs. A combined linkage map using the 666 SNPs shared by both populations resulted in a 1,078 cM map containing 302 non-redundant marker loci across 218 genotypes.

QTL Mapping Results:

Fhb1+ Population

Composite interval mapping identified 25 significant marker/trait associations spread across 13 chromosomes (Table 5 and Fig. 5) that clustered into 16 total QTL regions. A significance threshold of LOD = 3.2 was established by permutation testing to ensure excessive Type II errors were avoided. Although all traits exhibited significant genotype-by-environment interactions, QTL-by-environment interactions were only observed for the DON trait. QTL reported here were thus based on mean trait values across environments unless otherwise stated. No statistically significant epistatic QTL were identified.

The QTL peak identified on chromosome 1A was located at approximately 45 cM and was highly significant for variation in micro test weight (R^2 = 18%). A multi-trait peak on chromosome 1B is centered at 33 cM. Genes underlying that QTL region explained 11% of the variation in VSK, 11% of greenhouse FHB spread, and 15% of the differences in the 2011 DON accumulation.

Group 2 QTL were mapped on chromosomes 2A and 2D. The 2A QTL explained 10% of the variation in FHB severity, 9% of Type II greenhouse severity, and 9% of average DON accumulation and was placed on the most prominent 2A linkage group in the interval between 47 and 49 cM. There was one major QTL on the 2D chromosome at 13.8 cM that explained 40% of heading date variation and 15% of the 30 head weight variability.

Significant group 3 QTL were discovered on chromosomes 3A and 3B. The QTL for greenhouse resistance on 3A explained nearly 14% of variance in greenhouse FHB spread and was centered at 82.6 cM. The 3B QTL region occurred in the immediate neighborhood of the *Fhb1* gene at 2.2 cM, but only explained approximately 6% of FHB severity variance, and that was largely attributable to its association with the 2011 St. Paul environment.

Only a single QTL region was mapped to the group 4 chromosomes, a moderately significant peak located at 54 cM explaining 9% of the variation in incidence of FHB infection in the 2012 St. Paul environment.

Two significant QTL were identified on the group 5 homoeologs, with both on chromosome 5A. One was located at 20 cM and explained nearly 10% of the variation in incidence of FHB infection. The other occurred proximal to the first at 49 cM and accounted for 13% of VSK variability. Both of these QTL reside on the more marker dense 5A linkage group.

A large, single trait QTL was observed on chromosome 6A near the 50 cM position that explained 14% of field FHB severity, with the strongest environmental association coming from St. Paul in 2012. A second 6A QTL was mapped to the 13 cM position and explained 9% of FHB spread in the greenhouse. Although chromosome 6D was split into two linkage groups, and consisted of only 9 SNPs covering 26 total centiMorgans, a significant plant height QTL was mapped to the 7 cM region of the larger linkage group that accounted for 13% of plant height variance. The major group 6 finding was a large-

effect QTL influencing multiple traits in all environments located between 35 and 45 cM of chromosome 6B. This region of the genome displayed a major impact on resistance to FHB field severity (R^2 = 28%), FHB incidence (R^2 = 16%), micro test weight reduction (R^2 = 11%), 2012 St. Paul DON accumulation (R^2 = 10%), and mean DON accumulation (R^2 = 10%). The magnitude of effect on FHB severity, DON accumulation, and FHB incidence was the largest of all QTL identified for those traits.

There were two significant QTL regions observed on chromosome 7A, and one on 7B. The first 7A QTL was located on the distal end of the short arm at 2 cM and explained 12% of plant height variability. A second 7A QTL was at 77.5 cM and was very significant for resistance to DON accumulation (R^2 = 13.6%), but only in the 2012 St. Paul environment. The 7B QTL was mapped to the distal end of the long arm at 132 cM. It impacted DON accumulation, but was significant in only the 2011 St. Paul environment (R^2 = 9.4%).

***Fhb1*- Population**

Composite interval mapping identified twelve marker/trait associations across five chromosomes (Table 6 and Fig. 5) that clustered into six QTL regions. The significance threshold was again set at LOD = 3.2 and genotype-by-environment interactions were significant for the 2011-2012 environments, but QTL-by-environment interactions were only observed for DON accumulation. No statistically significant epistatic QTL were identified.

A highly significant QTL region was identified on chromosome 1B in both populations. That QTL region explained 12% of 2011 and 2012 heading date variation, 12% of the FHB severity variation across all environments, and 18% of the 2011 and 2012 VSK variance. The heading date association was equally present in both 2012 environments, FHB severity did not exceed marginal levels of significance in any single environment, and the VSK QTL was very significant in 2012 at both St. Paul and Crookston.

Two prominent QTL were mapped to the group 2 homoeologs. The 2A QTL explained 24% of the variance in DON concentration, with the only significant single-environment effect observed in the 2012 Crookston environment. The 2D QTL explained 32% of the heading date trait and 13% of 30 head weight. The heading date association was very strong in all environments, while the 30 head weight association was primarily due to a large effect in the 2011 St. Paul environment.

A single highly significant QTL region was found on chromosome 6B. This QTL explained 20% of FHB incidence in the 2012 St. Paul environment, 10% of DON accumulation across all environments, and 13% of 2011 and 2012 FHB severity. These findings are not the result of any single, highly influential environment. Although this QTL was associated with DON resistance in both populations, it was especially effective at reducing DON levels in the *Fhb1*- population.

There was one distinct QTL region mapped to chromosome 7A that explained 10% of the variance in plant height. No single, highly influential environment appeared responsible for the significance of this association.

Discussion

Phenotypes and Resistance Trait Correlation:

We studied multiple measures of FHB resistance in addition to heading date and plant height, two traits known to influence field-based FHB reaction. The field screening methodology for measuring FHB severity employed here serves as a good approximation for Type II resistance (r = 0.60), and previous studies have consistently implicated *Fhb1* in both resistance to FHB spread and DON accumulation. Resistance to FHB incidence (Type I or resistance to initial infection) has not been previously ascribed to *Fhb1* (Bai *et al.* 1999, Waldron *et al.* 1999, Liu and Anderson 2003), and its assessment in this study was limited because only the 2012 St. Paul environment produced infection levels low enough to recognize phenotypic differences in FHB incidence. Type II resistance as measured in the greenhouse was the trait most notably lacking a normal distribution (Table 3, Fig. 3). This could be due to single plant micro-environments only present in the greenhouse that prevented significant spread of infection. It had a moderately high coefficient of variation (55%), which was a result of the low trait mean. However, it also had high broad-sense heritability (h^2 = 0.89), indicating a high level of repeatability across greenhouse seasons, which along with check lines that met expectations indicated a high quality dataset. Heritability was similar to other greenhouse FHB experiments that ranged from 0.85-0.91 (Jiang and Ward 2006, Jiang *et al.* 2006, Li *et al.* 2012). While correlation with FHB severity in the field was high, 40% of the most resistant lines in the greenhouse (< 10% spread) were moderately susceptible (FHB severity >0.30) in field measurements. The lower level of disease observed in the greenhouse was likely the

result of the single infection point used in the greenhouse versus multiple infection points in the field. This finding suggests that some lines may possess poor Type I resistance, but are effective at preventing fungal spread (Type II resistance).

In field trials, where both populations were tested, we can observe the effect of *Fhb1*. Both populations exhibited the signs of polygenic resistance, but the *Fhb1*- population had a similar distribution pattern shifted toward the more susceptible values. Field traits were more normally distributed and showed much lower coefficients of variation than the greenhouse, but yielded lower broad sense heritabilities that were similar to other studies (Liu *et al.* 2005, Jiang *et al.* 2006, Li *et al.* 2012). These differences can be explained by the presence of more experimental units, but far greater environmental variability and genotype-by-environment interaction in the field trials. Trait distribution indicates that multiple resistance loci are impacting resistance, but *Fhb1* was observed to improve trait scores by 20-30%, consistent with previous findings (Pumphrey et al., 2007, Salameh *et al.* 2011, Balut *et al.* 2013).

Transgressive segregants were found on both sides of the distribution for all traits in the *Fhb1*+ population, while they only occurred relative to 'Bobwhite' in the absence of *Fhb1* (Fig. 3). This finding is also indicative of the polygenic control of FHB resistance traits. In a RIL population, where there is minimal dominance involved, there must be resistance loci present in 'Bobwhite' that combined with '260-2' alleles to enable the finding of greater levels of resistance than observed in the resistant parent, '260-2.'

The other interesting phenotypic finding involved trait correlations. Within–trait correlations were high across all resistance traits at r = 0.22-0.67. These were very similar to results obtained by Hao *et al*. (2012). Reductions in these correlations are indicative of differences in infection levels attributable to growing environment. St. Paul in 2011 and Crookston in 2012 had substantially higher levels of infection than the 2012 St. Paul location. This resulted in genotype-by-environment interactions that were biologically insignificant, based on the absence of QTL-by-environment interactions. The exception was the DON trait that displayed inconsistencies across environments.

Correlations between traits were also very robust, and usually present in the expected direction. The strongest positive correlations across environments existed between VSK and FHB severity and DON accumulation and FHB severity, while the highest magnitude negative correlations occur between 30 head weight and FHB severity and 30 head weight and VSK. These results are similar to previous findings (Suzuki *et* al. 2012, Tamburic-Ilincic 2012). An exception was found with 2011 DON. 2012 was hot and dry across the midwest, with average temperatures during the period of disease development being 2 °C higher than in 2011 (Minnesota Department of Natural Resources 2016). While overall DON levels in St. Paul were similar in both years, the weather differences could have led to the correlation inconsistencies and caused the 2011 DON results to deviate from both 2012 environments. Heading date was correlated with 30 head weight and DON accumulation in the opposite direction as expected, indicating that in this study, earlier heading date was advantageous, though not for measures of Type II resistance.

Correlation between resistance traits and plant height was in the expected direction, but magnitudes were very low, indicating little plant height effect on disease resistance.

Linkage Maps:

Linkage mapping generated 23 distinct linkage groups (Fig. 4). Of the 1,650 polymorphic SNPs mapped, 78% of them mapped to redundant locations, leaving 352 unique marker loci. While this percent redundancy was high, it is similar to biparental studies using the 9K SNP chip that found approximately 70% and 53% cosegregating SNPs (Li *et al.* 2013 and Zurn *et al.* 2014, respectively). Since QTL mapping using recombinant inbred lines doesn't allow for heterozygous loci (Wang *et al.* 2007), any datapoints marked as heterozygous were removed. That missing data added to the number of undefined genotype calls, and increased the occurrence of redundant markers.

With a total genetic distance of 1,565 cM, the map size is a relatively standard biparental map size for wheat (Somers *et al.* 2004). While the D genome was underrepresented in this study, this is not atypical. Cavanagh *et al.* (2013) mapped only 8% of the loci on the 9K Illumina chip to the D genome, indicating an overall lack of diversity in the D genome as compared to the A and B genomes. We mapped only five polymorphic SNPs to chromosome 4D, which did not allow the formation of a linkage map for this chromosome. Chromosomes 3D, 5D, and 7D yielded only 39 polymorphic SNPs and combined for a total of 7 unique datapoints, but small linkage groups were generated for these chromosomes. We were able to generate a map of chromosome 6D, which is known to have low diversity and sparse marker coverage, particularly in the proximal

regions (Akhunov *et al*. 2010). The dearth of mapped FHB resistance QTL in the D genome (Buerstmayr *et al*. 2009) indicates either an inability to detect new QTL due to lack of marker coverage, a lack of native resistance in the D genome donor, *Aegilops tauschii*, or an inability to express some *Ae. tauschii* resistance genes in hexaploid wheat.

Although the original simple inheritance hypothesis did not require resource allocation for genotyping the *Fhb1*- population, it took on greater importance following the discovery of polygenic inheritance. The preliminary map created by Xin Li (unpublished) has a total genetic distance of approximately 1,744 cM. A total of 666 of the 782 total *Fhb1*- SNPs were shared among the two mapping populations, allowing enough common markers to provide confidence that the same QTL region was mapped in each population. The joint linkage map using these shared loci did not yield new QTL results and had 600 fewer cM and fewer loci than either individual population map.

QTL for *Fusarium* Head Blight Resistance:

The most significant findings from this study are the identification of multiple FHB resistance and correlated trait QTL (Tables 5 and 6, Fig. 5), and a lack of evidence that any of these are clear inhibitors of *Fhb1*. While few QTL were discovered in all environments, those that were identified in multiple environments were also significant in the combined analysis over environments. With the exception of DON QTL, none of which were significant in more than one environment, any loci not found in the combined analysis represent possible false positives and were not investigated further.

One major effect QTL for heading date was found on the short arm of chromosome 2D. This QTL was also significant for 30 head weight, but was otherwise not associated with other measures of FHB resistance. Although the methodology for assessing heading date employed in this study confounded heading with anthesis, the effect of the 2D QTL was highy significant in all environments (LOD > 10) and both populations. This 2D heading date QTL is in the same genomic region as the major photoperiod sensitivity gene *Ppd-D1* (Hanocq *et al.* 2004, Beales *et al*. 2007). The large effect is indicative of the gene and the QTL peak occured at the SNP *wsnp_CAP12_c812_428290*, which has been mapped within 1cM of the SNP flanking *Ppd-D1* (Perez-Lara *et al*. 2016). The Bobwhite allele confers the equivalent of two days earlier heading across all environments and results in almost one gram improvement in 30 head weight, making it the desirable allele at this locus.

The other agronomic trait measured was plant height, and significant QTL were identified on chromosomes 6D and 7A. Both QTL were mapped from the St. Paul environments, while the Crookston environment produced plants on average two centimeters shorter than St. Paul and with less trait variability. QTL mapping likely failed to identify any plant height associations in the Crookston data because of the lack of variation in this dataset. The location of the 7A QTL is similar in location and effect to the one reported by Klahr *et al*. (2007). Both regions are not coincident with any disease resistance traits, meaning no height or heading date QTL exhibit a correlated effect on direct measures of FHB resistance.

The 30 head weight trait isn't a direct measure of resistance or susceptibility, but it is an indicator of kernel abortion and seed condition (i.e. seed size or degree of seed shriveling). Since the present study didn't indicate quantifiable levels of kernel abortion, the trait is likely not critical for understanding FHB resistance in this population, but does provide support for other QTL. A single major 30 head weight QTL was identified on chromosome 2D in the region of *Ppd-D1*. An epistatic interaction between *Ppd-D1* and a QTL on 4D for kernel weight in 'Ernie' and 'Massey' (Liu *et al*. 2013) indicates that the 2D 30 head weight QTL could be a pleiotropic effect of *Ppd-D1*. Although *Ppd-D1* may not directly confer FHB resistance, the presence of this 30 head weight QTL in both populations confirms the strong pleiotropic effect on FHB-related traits.

Micro test weight also measures the ability of the host plant to produce plump, sound grain. Micro test weight was measured as a bulk of seeds across replications in both 2012 locations, but only the Crookston environment produced sufficient variability for mapping. The highly significant 1A QTL found here is in a similar location to a FHB spread in the spike and DON content QTL mapped in the line 'CJ 9306' (Jiang *et al*. 2007a,b). Lack of association with other traits and the 'Bobwhite' source of resistance ultimately diminish the value of this QTL. The other moderate effect QTL on chromosome 6B has a lesser effect in a QTL region that displays larger effects for other FHB resistance traits.

We mapped QTL for FHB incidence from the 2012 St. Paul environment on chromosomes 4A, 5A, and 6B. The 5A QTL is located in a similar region to *Fhb5* mapped in 'Wangshuibai' (Xue *et al*. 2011), with a similar effect. However, this QTL

was not coincident with any other trait examined and was not observed in the *Fhb1*- population. Pedigree analysis did not indicate the presence of 'Wangshuibai' in the population, though this QTL has been identified in other backgrounds containing 'Sumai 3' (Buerstmayr *et al*. 2009). The 6B QTL was coincident with FHB severity, micro test weight, and DON resistance traits and is located in the same region as *Fhb2* (Cuthbert *et al*. 2007). The 6B QTL was also mapped in the *Fhb1*- population, and thus regardless of *Fhb1* status, the '260-2' allele confers a 5% improvement in FHB incidence.

Visually scabby kernel ratings are the most direct measure of seed quality, and serve as a proxy for FHB severity and resistance to DON accumulation. We identified a moderate effect QTL on 5A and another on 1B in which the 'Bobwhite' allele conferred resistance in both populations. QTL in this region of 1B for FHB severity have been previously identified in the susceptible cultivars 'Alondra' and 'Lynx' (Zhang *et al*. 2004 and Schmolke *et al*. 2005, respectively).

While there were QTL for FHB severity identified on four of the 24 linkage groups, the 2A and 6B QTL exhibit the largest effect in the presence of *Fhb1* with the resistance conferred by '260-2.' The 2A severity QTL displayed a lesser effect and was insignificant ($p = 0.06$) in the *Fhb1*- population when analyzing single marker effects (Table 8), but improved FHB severity by 2% when the '260-2' allele was present along with *Fhb1*. The 2A QTL was identified in the higher disease environments of 2011 St. Paul and 2012 Crookston. Initial *Fhb1* mapping work identified a QTL on 2AL in the susceptible variety 'Stoa' (Waldron *et al*. 1999, Anderson *et al*. 2001). A QTL in the same location was also found in the moderately resistant variety 'Renan' (Gervais *et al*.

2003). The 6B QTL showed a very consistent and sizable effect in all environments examined, explaining 28% of the variance in FHB severity, and was mapped to the same location as *Fhb2* (Waldron *et al.* 1999, Anderson *et al.* 2001, Cuthbert *et al.* 2007). This QTL appears less impactful in the absence of *Fhb1*, but still improves FHB field severity by greater than 3%.

In contrast to many of the traits discussed, resistance to the accumulation of the mycotoxin DON is a direct measure of the effect on grain quality and represents much of the economic impact of FHB. It is also hypothesized to be closely associated with the *Fhb1* gene, which has been reported to act as a mycotoxin detoxifier (Lemmens *et al.* 2005). There were QTL for DON accumulation mapped on the 2A and 6B chromosomes in the combined analysis, and additional ones on 1B, 7A, and 7B in individual environment analyses. The QTL on 2A and 6B are in the same locations as previously discussed for improved FHB severity, with the 6B QTL identified as significant in the 2012 St. Paul location. Both the 2A and 6B QTL were also highly significant in the *Fhb1*- population, especially the 2AL region, which reduced DON concentration by 2.5 ppm in the 2012 Crookston environment. A substantial effect in the 2012 St. Paul environment was contributed by the 7A QTL, but the region was insignificant for all other traits. The 1B QTL showed the largest effect in the 2011 St. Paul environment, and was coincident with the QTL for VSK. Like this VSK QTL, the source of resistance for the 7B DON QTL was 'Bobwhite.' No previous study has implicated any region of chromosome 1B in mycotoxin resistance. The absence of an effect from the *Fhb1*+ QTL in the severely infected 2012 Crookston environment indicates these regions may be

overwhelmed under high inoculum loads and environmental conditions highly favorable to *Fusarium* infections.

Given that mycotoxin resistance leads to improved Type II resistance, mapping QTL for FHB spread following point inoculation in the greenhouse can act as a cumulative measure of *Fhb1*-associated resistance. This was the one trait evaluated in a controlled environment and the mapping analysis identified significant QTL on chromosomes 1B, 2A, 3A, and 6A. The 3A and 6A QTL were not coincident with any field-based FHB resistance traits. While the 1B QTL was highly significant, it was located in the VSK QTL region previously discussed, with resistance conferred by 'Bobwhite.' The 2A QTL region improved resistance to FHB spread by nearly 5%, with the effect conferred by the '260-2' allele. This important QTL is in the same region of chromosome 2AL identified for FHB severity and reduced DON accumulation.

Fhb1 Inhibition:

The hypothesis under investigation in this study involved the possible presence of a genomic region that may be suppressing *Fhb1* and lead to a reduction in FHB resistance. The initial cloning study indicated simple inheritance, which led to the hypothesis that *Fhb1* inhibition was caused by a small number of genes with large effects (Liu *et al.* 2008). However, that study did not aim to characterize inhibition or investigate regions of the genome other than *Fhb1*, which led to the alternative hypothesis of polygenic inheritance, with some loci possibly acting independently of *Fhb1*. Results of the current study reject the simple inheritance hypothesis in favor of the polygenic model.

An inhibitor target was defined as a locus whose resistant allele for all associated traits was contributed by '260-2' and displayed association with multiple disease resistance traits, at least one of which must be the *Fhb1*-related traits of spread within a spike, FHB severity in the field, or DON accumulation. If such a QTL was behaving as a suppressor of *Fhb1*, the trait effect would not be observed in the *Fhb1*- population (Fig. 2). This uncovered two major genomic regions that exhibit a strong and consistent effect across multiple resistance traits.

The most prominent and consistent QTL identified is the one located on chromosome 6B. This QTL's chromosomal location, trait associations, and effects are consistent with the previously mapped QTL *Fhb2* (Waldron *et al*. 1999, Anderson *et al*. 2001, and Cuthbert *et al*. 2007). A microsatellite marker screen on the NIL pair and relevant controls using the tightly linked GWM644 marker (Cuthbert *et al*. 2007) confirmed the identity of this QTL as *Fhb2*, which was not selected against during the process of NIL development (Table 7). The combination of resistant genotypes at both *Fhb1* and *Fhb2* has been found to cause a 35% reduction in FHB severity, as opposed to a 26% reduction when only *Fhb1* was present in its homozygous resistant state (Shen *et al*. 2003). In the current study, all mapping lines were homozygous for the presence of *Fhb1*, and lines possessing *Fhb2* exhibited an 18% improvement in FHB severity, 25% less DON content, and 36% less spread of symptoms following point-inoculation in the greenhouse, than their non-*Fhb2* counterparts. The 6B region also displayed a highly significant effect on DON accumulation (R^2 = 10.2% overall), FHB incidence (R^2 = 19.8%), and FHB severity (R^2 = 13.1%) in the *Fhb1*- population. Lines possessing *Fhb2* showed a 15% reduction in FHB

severity and a 12% reduction in DON concentration than those lacking *Fhb2*. The effect of the substitution of a 'Bobwhite' allele for a '260-2' allele was nearly 4% fewer infected spikelets and a 0.87 ppm lower DON content. These effects indicate that the *Fhb2* gene is not suppressing the effect of *Fhb1*, but rather is contributing to greater FHB resistance, either with or without *Fhb1* being present. The association with DON accumulation was highly significant in 2012 St. Paul, but not in the higher infection environments, indicating that *Fhb2* may code for local defense genes (Kosaka *et al*. 2015a, Kosaka *et al*. 2015b) that are highly effective in low disease conditions, but ineffective at preventing epidemics when the disease is prevalent and severe.

The other major QTL identified here is the one located on the long arm of chromosome 2A. While Waldron *et al*. (1999) and Anderson *et al*. (2001) mapped a QTL (*Qfhb.ndsu-2AL*) in a similar region in 'Stoa,' analysis of check lines with both the most significant SNPs from the *Fhb1+* population and microsatellites linked to the previously mapped region indicated that the QTL observed here is likely not identical, regardless of the fact that '260-2' is descended from 'Stoa' (Table 7). Lack of concordance between these markers in a randomly genotyped RIL subset supports this conclusion (suppl. Table 3), regardless of comparisons across marker platforms and lack of recombination resolution in the original study. The 'Stoa' QTL explained 14% of disease severity variation in the 'Sumai 3' studies (Waldron *et al*. 1999, Anderson e*t al*. 2001). In the current study, lines in the *Fhb1+* mapping population that were homozygous for the presence of the 2A QTL showed a 15% reduction in FHB severity, 26% less DON content, and 47% less FHB spread in the greenhouse than lines lacking this QTL. Single marker analysis on the

Fhb1- population (Table 8) showed the QTL had a large effect on DON accumulation (R^2 = 9.6%), but only a marginal effect on FHB severity (R^2 = 3.9%). In lines without *Fhb1*, there was an 8% improvement in FHB severity and a 16% reduction in DON accumulation. The effect of replacing a 'Bobwhite' allele with a '260-2' allele at this QTL was 2.2% fewer infected spikelets in the field, 4.6% less disease spread in the greenhouse, and nearly 1 ppm lower DON in grain. Impact of the 2AL QTL on FHB severity and spread in a head is very similar to that of *Qfhb.ndsu-2AL*. Previous studies involving this QTL have not measured DON, and the presence of stronger resistance than other QTL identified on chromosome 2A further suggests that this could be a novel QTL. The absence of an association with FHB severity in the *Fhb1*- population indicates this QTL could be inhibiting the anti-fungal activity of the cloned pore-forming toxin-like gene (Rawat *et al*. 2016). Since the initial cloning study (Liu *et al*. 2008) didn't measure DON accumulation, and DON detoxification in the *Fhb1* interval is not controlled by the cloned gene, the lack of a similar inhibitory effect on DON accumulation doesn't preclude the 2A QTL from being a *Fhb1* inhibitor. However, a single field-based trait doesn't provide sufficient confidence to make an inhibitor declaration either. Further research will be required to generate greenhouse data and recombinants in this 2A QTL region that can enable a more convincing conclusion regarding resistance gene inhibition.

Our results don't indicate the presence of an obvious inhibitor of *Fhb1*. No previous studies have tested for resistance to DON accumulation conferred by *Fhb2*, although several candidate genes were identified using flanking marker sequences and transcriptomic analysis that may aid in the detoxification of DON (Dhokane *et al*. 2016).

The significant effect of *Fhb2* in the absence of *Fhb1* indicates that this is likely a local defense gene such as those involved in cell wall defense, including peroxidases and proteases, which are thought to be expressed later in the infection than DON detoxification genes (Kosaka *et al*. 2015a, Kosaka *et al*. 2015b). While there is evidence that the 2A QTL may interact with *Fhb1*, we conclude that it is an independent FHB resistance QTL because it does not match the definition of an inhibitor. Until further research can provide a definitive conclusion about the 2A QTL, selection for the '260-2' allele at *wsnp_Ku_c16522_25425455* on 6B and *wsnp_Ex_c12219_19526749* on 2AL will allow for improved FHB resistance during variety development (Anderson 2007), especially in the presence of *Fhb1*.

QTL Mapping Methodology:

This study represents the first instance of QTL mapping performed in populations known to be homozygous for the presence or absence of a major-effect QTL. Conventional mapping has focused on using parents with highly contrasting phenotypes and resulting populations segregating for major genes. That strategy results in difficulty mapping minor-effect QTL, unless population sizes are exceedingly large (>150 genotypes). The populations used in this study were homozygous for either the presence or absence of *Fhb1*, thus removing its potential to mask minor-effect loci.

The *Fhb1+* population identified six QTL regions for resistance not found in the *Fhb1-* population and not previously mapped (Buerstmayr *et al*. 2009). These included a QTL for resistance to initial infection on 4A, a VSK QTL on 5A, DON concentration QTL on

7A and 7B, a FHB severity QTL on 6A, and QTL for resistance to spread in the head on 3A and 6A. The R^2 values for these QTL ranged from 9% for the 4A incidence QTL to 14% for the 6A severity QTL. None of these population-specific QTL displayed significance in the highest infection environment (2012 Crookston), but appeared effective under lower FHB pressure as in the two St. Paul environments.

The *Fhb1*- population identified one QTL region not found in the *Fhb1*+ population and not previously mapped (Buerstmayr *et al.* 2009), as well as unique trait associations for regions identified in the presence of *Fhb1*. These include FHB severity QTL on 1B and 2D, and a heading date QTL in the same 1B region. R^2 values for these QTL ranged from nearly 12% for the 1B region, to 16% for the 2D FHB severity QTL. None of these QTL displayed environment-specific effects, and they only achieved significance in the combined-environment analysis.

While the *Fhb1*+ population identified more QTL than the *Fhb1*- population, this could be due to small phenotypic differences discovered using a slightly larger population size, spatial trait adjustments, and greater replication. However, the population lacking *Fhb1* could be used to identify smaller-effect QTL and resistant loci from the susceptible parent. While trait associations likely involving the major genes *Fhb2* and *Ppd-D1* were stronger when *Fhb1* was present (severity R^2 = 28% (*Fhb1*+) versus R^2 = 13% in *Fhb1*-, and heading date R^2 = 40% (*Fhb1*+) versus R^2 = 32% in *Fhb1*-), it was easier to identify lesser-effect and possibly novel QTL on 1B and 2A when *Fhb1* was not present to mask the effect (VSK R^2 = 18% (*Fhb1*-) versus R^2 = 11% in *Fhb1*+, and DON R^2 = 24% (*Fhb1*-) versus 9% in *Fhb1*+). In the absence of *Fhb1*, '260-2' becomes equivalent to

'260-4,' which means the *Fhb1*- population was equivalent to a population derived from moderately susceptible parents. *Fhb1*+ population-specific QTL were detected only in the two St. Paul environments, with little effect evident in the highly infectious conditions while in the presence of a major resistance gene. The only QTL found in the *Fhb1*- population in the highly favorable 2012 Crookston environment was for resistance to DON accumulation, meaning the effects of these QTL were otherwise easily overwhelmed by the fungus in these conditions. While high-infection environments are best for mapping large-effect genes, it appears the detection of minor-effect loci is aided by the use of more moderate disease pressure.

Small population sizes are inadequate for reliably identifying QTL and estimating effect magnitudes of small-effect loci (Beavis 1998, Vales *et al*. 2005). When mapping loci for FHB resistance with conventional methods, populations with less than 100 genotypes are unable to detect anything other than large-effect loci (Buerstmayr *et al*. 2009). However, when large-effect loci are no longer segregating, the significance of small-effect loci improves. We were able to identify novel QTL from the 'Sumai 3'-derived parent in each population that were not previously mapped in 'Sumai 3' studies segregating for *Fhb1*. A screen with the most significant SNPs indicates the 2A QTL is present in 'Sumai 3,' yet was not mapped in previous studies, possibly due to a masking effect from the segregating *Fhb1* locus. A well-designed phenotyping strategy in a population derived from moderately susceptible parents should enable detection of small-effect resistance genes that can generate markers for MAS, without the need for a large and inefficient population size.

Table 1: Phenotypic correlations among FHB related traits in the *Fhb1+* population, based on means across replications. Bold values indicate statistically significant correlations ($p < 0.05$).

	2011 StP Heading	2012 StP Heading	2012 Crk Heading	2011 Height	2012 StP Height	2012 Crk Height	2011 30 Hd Wt.	2012 StP 30 Hd Wt.	2012 Crk 30 Hd Wt.	2011 VSK	2012 StP VSK	2012 Crk VSK	2012 StP Test Weight	2012 Crk Test Weight	2011 Severity	2012 StP Severity	2012 Crk Severity	2011 DON	2012 StP DON	2012 Crk DON	Greenhouse
2011 StP Heading	1																				
2012 StP Heading	**0.51**	1																			
2012 Crk Heading	**0.51**	**0.74**	1																		
2011 Height	0.04	0.08	0.08	1																	
2012 StP Height	0.13	**0.22**	**0.21**	**0.81**	1																
2012 Crk Height	-0.02	0.04	-0.05	**0.76**	**0.71**	1															
2011 30 Hd Wt.	**-0.29**	**-0.46**	**-0.43**	**0.21**	0.05	0.11	1														
2012 StP 30 Hd Wt.	**-0.17**	**-0.36**	**-0.30**	**0.26**	**0.28**	**0.23**	**0.44**	1													
2012 Crk 30 Hd Wt.	-0.13	**-0.20**	**-0.20**	0.04	0.07	0.09	**0.57**	**0.47**	1												
2011 VSK	-0.01	**0.17**	0.13	-0.07	-0.05	-0.04	**-0.45**	**-0.28**	**-0.42**	1											
2012 StP VSK	-0.04	0.04	0.07	-0.14	-0.11	-0.13	**-0.29**	**-0.45**	**-0.33**	**0.46**	1										
2012 Crk VSK	**-0.19**	**-0.21**	-0.11	**-0.17**	**-0.25**	-0.14	**-0.20**	**-0.33**	**-0.33**	**0.22**	**0.48**	1									
2012 StP Test Weight	-0.15	**-0.34**	**-0.31**	-0.03	0.04	0.02	**0.32**	**0.65**	**0.29**	**-0.18**	**-0.51**	**-0.27**	1								
2012 Crk Test Weight	-0.12	-0.05	**-0.21**	-0.10	-0.06	-0.06	**0.40**	**0.20**	**0.45**	**-0.21**	**-0.25**	**-0.57**	**0.39**	1							
2011 Severity	0.01	0.10	0.06	-0.10	-0.08	0.05	**-0.57**	**-0.27**	**-0.49**	**0.49**	**0.40**	**0.39**	**-0.25**	**-0.45**	1						
2012 StP Severity	0.14	**0.33**	**0.20**	-0.09	-0.17	-0.02	**-0.30**	**-0.52**	**-0.36**	**0.30**	**0.34**	**0.21**	**-0.35**	-0.12	**0.54**	1					
2012 Crk Severity	**-0.23**	**-0.19**	**-0.21**	-0.08	-0.11	-0.04	**-0.27**	-0.16	**-0.42**	**0.31**	**0.37**	**0.51**	-0.16	**-0.48**	**0.67**	**0.38**	1				
2011 DON	-0.05	0.16	0.12	0.06	**0.19**	0.08	-0.09	0.03	-0.08	**0.40**	0.13	-0.09	0.02	-0.05	**0.23**	0.05	**0.21**	1			
2012 StP DON	0.09	**0.21**	**0.23**	-0.07	-0.01	-0.07	**-0.32**	-0.14	**-0.30**	**0.28**	**0.31**	0.10	-0.02	**-0.25**	**0.44**	**0.40**	**0.41**	**0.56**	1		
2012 Crk DON	0.09	0.17	**0.28**	-0.03	0.07	-0.07	**-0.36**	-0.15	**-0.32**	**0.27**	**0.28**	**0.29**	**-0.18**	**-0.56**	**0.47**	**0.17**	**0.42**	**0.38**	**0.53**	1	
Greenhouse	0.07	0.01	0.05	-0.03	-0.05	-0.07	**-0.18**	**-0.19**	**-0.36**	**0.24**	**0.29**	**0.28**	**-0.24**	**-0.31**	**0.52**	**0.46**	**0.47**	0.15	**0.37**	**0.40**	1

Table 2: Phenotypic correlations of FHB related traits in the *Fhb1+* population, based on trait means across environments. Bold values indicate statistically significant correlations ($p < 0.05$).

	Greenhouse	Heading	Height	30 Hd Wt	Test Weight	VSK	DON	Severity	Incidence
Greenhouse	1								
Heading	0.05	1							
Height	-0.06	0.10	1						
30 Hd Wt	**-0.29**	**-0.41**	**0.21**	1					
Test Weight	**-0.31**	**-0.19**	-0.08	**0.43**	1				
VSK	**0.36**	0.02	**-0.17**	**-0.55**	**-0.48**	1			
DON	**0.42**	**0.27**	0.00	**-0.33**	**-0.50**	**0.38**	1		
Severity	**0.60**	0.03	-0.09	**-0.54**	**-0.46**	**0.58**	**0.48**	1	
Incidence	**0.35**	**0.22**	**-0.23**	**-0.44**	**-0.18**	**0.36**	**0.41**	**0.59**	1

Table 3: Trait summaries showing ANOVA results, broad sense heritabilities, and coefficients of variation for the combined population and Pearson Chi-squared normality tests for each separate population. *Fhb1+* = population homozygous for *Fhb1* presence and *Fhb1-* = population homozygous for *Fhb1* absence

	DF	SS	MS	F-value	p-value	H^2	C.V. (%)	*Fhb1+* Normality	*Fhb1-* Normality
Heading Date								X^2 p-value	X^2 p-value
Genotype	225	301.05	1.34	5.43	<0.001	0.86	9.3	0.063	0.003
Environment	2	111.49	55.74	226.14	<0.001				
GxE	450	110.93	0.25	2.45	<0.001				
Replicate(Env)	4	0.68	0.17	1.70	0.148				
Residuals	771	77.50	0.10						
Plant Height									
Genotype	225	24422.74	108.55	7.96	<0.001	0.89	4.0	0.379	<0.001
Environment	2	7244.99	3622.49	265.53	<0.001				
GxE	450	6139.06	13.64	1.99	<0.001				
Replicate(Env)	4	252.71	63.18	9.22	<0.001				
Residuals	770	5277.06	6.85						
Test Weight									
Genotype	219	186.50	0.85	3.03	<0.001	0.31	5.5	0.749	0.601
Environment	1	364.62	364.62	1295.40	<0.001				
Residuals	206	57.98	0.28						
30 Head Weight									
Genotype	225	11344.47	50.42	4.29	<0.001	0.81	23.8	0.417	0.142
Environment	2	9912.25	4956.12	421.40	<0.001				
GxE	450	5292.48	11.76	1.68	<0.001				
Replicate(Env)	4	229.78	57.44	8.23	<0.001				
Residuals	773	5396.13	6.98						
Incidence									
Genotype	225	5.12	0.02	1.83	<0.001	0.66	10.6	<0.001	<0.001
Environment	2	3.84	1.92	154.21	<0.001				
GxE	450	5.60	0.01	2.16	<0.001				
Replicate(Env)	4	0.26	0.06	11.14	<0.001				
Residuals	781	4.50	0.01						
VSK									
Genotype	225	168700.90	749.78	3.77	<0.001	0.83	13.9	0.003	0.174
Environment	2	296464.22	148232.11	744.61	<0.001				
GxE	450	89583.56	199.07	4.57	<0.001				
Replicate(Env)	4	5165.82	1291.46	29.66	<0.001				
Residuals	773	33656.14	43.54						

	DF	SS	MS	F-value	p-value	H^2	C.V. (%)	*Fhb1+* Normality	*Fhb1-* Normality
DON								X^2 p-value	X^2 p-value
Genotype	225	5979.09	26.57	2.13	<0.001	0.75	19.6	0.011	0.519
Environment	2	8616.17	4308.09	345.16	<0.001				
GxE	450	5616.57	12.48	7.10	<0.001				
Replicate(Env)	2	52.64	26.32	14.97	<0.001				
Residuals	342	601.35	1.76						
FHB Severity									
Genotype	225	16.23	0.07	2.76	<0.001	0.73	38.4	0.157	0.384
Environment	2	16.00	8.00	306.44	<0.001				
GxE	450	11.75	0.03	1.73	<0.001				
Replicate(Env)	4	0.25	0.06	4.07	0.003				
Residuals	773	11.68	0.02						
Greenhouse									
Genotype	119	25.32	0.21	7.36	<0.001	0.89	55.0	<0.001	
Environment	1	2.33	2.33	80.39	<0.001				
GxE	119	3.44	0.03	2.09	<0.001				
Replicate(Env)	6	0.63	0.10	7.54	<0.001				
Residuals	713	9.87	0.01						

Heading Date = date of 50% anthesis
Plant Height = distance from soil to top of the spike (cm)
Test Weight = grain weight in a 15.7 mL vessel (g)
30 Head Weight = weight of 30 primary spikes (g)
Incidence = fraction of spikes displaying disease symptoms in the field
VSK = percentage of visually scabby kernels in a grain sample
DON = deoxynivalenol concentration of a grain sample (ppm)
FHB Severity = fraction of spikelets displaying disease symptoms per spike in the field
Greenhouse = fraction of spikelets displaying symptoms of disease spread

Table 4: Number of mapped SNPs assigned to consensus chromosomes (Cavanagh *et al*. 2013) in the *Fhb1+* population homozygous for the presence of *Fhb1* and the *Fhb1-* population homozygous for the absence of *Fhb1*.

Chromosome	SNPs in *Fhb1* + Population	SNPs in *Fhb1* - Population
1A	127	51
1B	92	27
1D	21	17
2A	63	35
2B	165	6
2D	37	24
3A	112	58
3B	125	69
3D	9	6
4A	22	22
4B	43	26
4D	5	2
5A	147	99
5B	145	95
5D	21	5
6A	85	58
6B	73	38
6D	8	3
7A	129	69
7B	108	59
7D	9	13
Unknown	104	0

Table 5: Significant (LOD > 3.2) QTL for FHB-related and agronomic traits in the *Fhb1+* RIL population homozygous for the presence of *Fhb1* (260-2/Bobwhite). Trait values were combined across environments unless otherwise noted. Table shows chromosome identity, location in centiMorgans, significance (LOD), % variance explained (R^2), and the phenotypic effect of the 'Bobwhite' allele.

Trait	Chromosome	Location (cM)	LOD	R^2	Effect of 'Bobwhite' Allele
FHB Incidence-2012-St. Paul	4A	53.8	3.4	9.0	4.29%
FHB Incidence-2012-St. Paul	5A-1	20.3	3.6	9.6	4.59%
FHB Incidence-2012-St. Paul	6B	44.6	5.6	15.5	5.83%
Heading Date	2D	13.8	14.9	40.1	-2 days
Plant Height	6D-2	7.0	3.7	12.8	1.16 cm
Plant Height	7A	1.9	4.4	12.4	-1.16 cm
Micro Test Weight-2012-Crookston	1A	44.7	6.3	17.9	0.36 g
Micro Test Weight-2012-Crookston	6B	34.8	3.7	10.9	-0.26 g
30 Head Weight	2D	13.8	5.4	15.4	0.86 g
VSK	1B	33.1	4.0	11.1	-2.28%
VSK	5A-1	49.1	4.0	13.1	2.39%
DON Concentration-2011-St. Paul	1B	33.1	5.6	14.9	0.58 ppm
DON Concentration-2011-St. Paul	7B	131.9	3.7	9.4	-0.45 ppm
DON Concentration-2012-St. Paul	6B	43.6	3.5	10.0	0.51 ppm
DON Concentration-2012-St. Paul	7A	77.5	4.4	13.6	0.61 ppm
DON Concentration-Mean	2A-1	49.1	3.4	8.6	0.82 ppm
DON Concentration-Mean	6B	43.6	4.0	10.3	0.87 ppm
FHB Severity	2A-1	49.1	5.0	9.7	2.16%
FHB Severity	3B	2.2	3.4	5.6	3.71%
FHB Severity	6A	50.6	6.7	14.0	-2.61%
FHB Severity	6B	43.1	12.2	27.8	3.65%
FHB Spread-Greenhouse	1B	37.0	3.8	11.1	-5.29%
FHB Spread-Greenhouse	2A-1	47.7	3.5	8.5	4.61%
FHB Spread-Greenhouse	3A	82.6	3.7	13.9	5.86%
FHB Spread-Greenhouse	6A	12.9	3.3	9.3	4.79%

Table 6: Significant (LOD>3.2) QTL for FHB-related and agronomic traits in the *Fhb1*- RIL population homozygous for the absence of *Fhb1* (260-2/Bobwhite). Traits are combined across 2011 and 2012 environments unless otherwise noted. Table shows chromosome identity, location in centiMorgans, significance (LOD), % variance explained (R^2), and the phenotypic effect of the 'Bobwhite' allele.

Trait	Chromosome	Location (cM)	LOD	R^2	Effect of 'Bobwhite' Allele
FHB Incidence-2012-St. Paul	6B	43.2	3.9	19.8	4.90%
VSK	1B	34	5.4	18.1	-3.74%
DON Concentration-2012-Crookston	2A	50.5	5.3	22.0	2.54 ppm
DON Concentration	2A	54.3	5.0	23.6	1.16 ppm
DON Concentration	6B	38.3	3.3	10.2	0.65 ppm
FHB Severity	2D	74.2	3.5	16.0	-3.85%
FHB Severity	6B	40.2	4.4	13.1	3.35%
FHB Severity	1B	30.4	4.1	11.9	-3.40%
Heading Date	1B	37.7	4.6	11.7	1 day
Heading Date	2D	12.8	10.6	31.8	-1.5 days
Plant Height	7A	79.9	3.5	9.8	-1.28 cm
30 Head Weight	2D	13.8	3.2	12.8	0.92 g

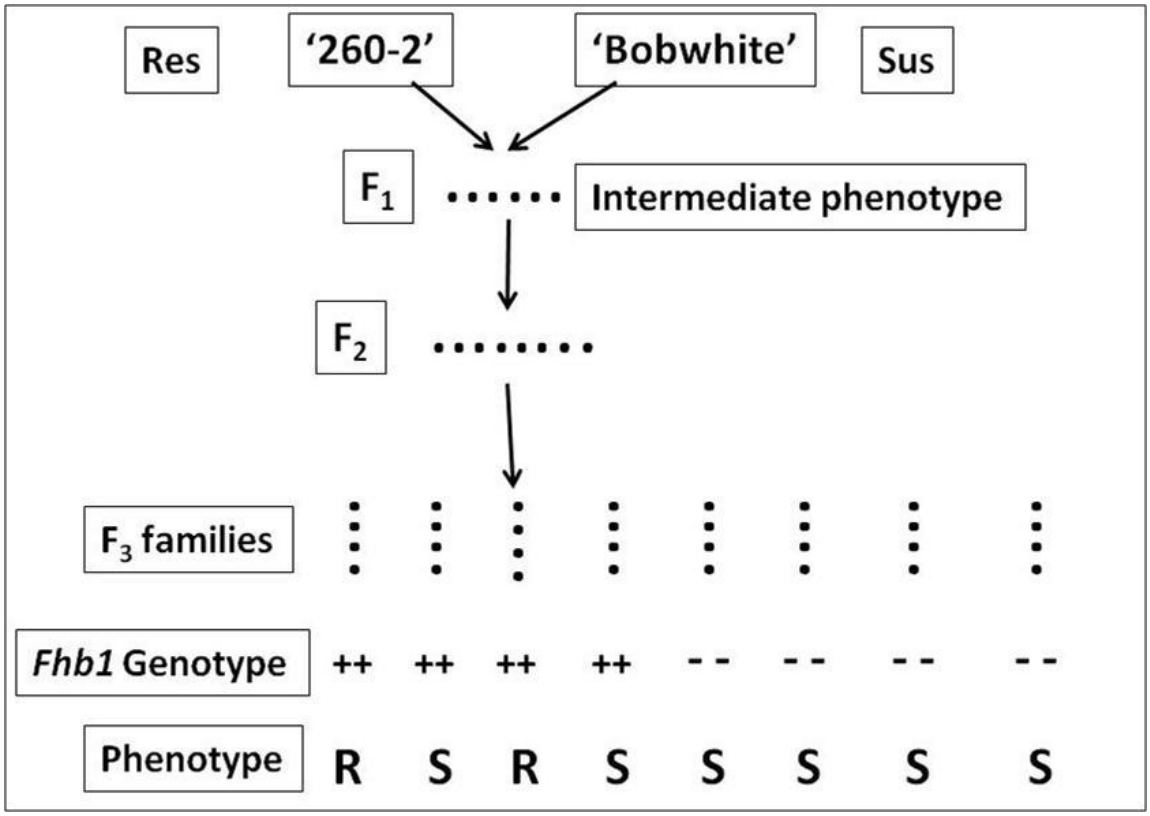
Table 7: Marker analysis comparing the genotype of *Fhb1* NILs to key controls at the *Fhb1* marker (UMN10), the *Fhb2* marker (GWM644), and significant markers linked to the 2A QTL (SNP #6753 & #1597). '260-2' indicates the resistant allele and 'Bobwhite' indicates the susceptible allele.

	UMN10	GWM644	SNP #6753	SNP #1597
Genotype	***Fhb1***	***Fhb2***	**2A QTL**	
260-2	260-2	260-2	260-2	260-2
Bobwhite	Bobwhite	Bobwhite	Bobwhite	Bobwhite
260-4	Bobwhite	260-2	260-2	260-2
Wheaton	Bobwhite	Bobwhite	Bobwhite	Bobwhite
Norm	Bobwhite	Bobwhite	Bobwhite	Bobwhite
Sumai 3	260-2	260-2	260-2	260-2
Stoa	Bobwhite	Bobwhite	260-2	Bobwhite

Table 8: Effect of significant markers from the *Fhb1+* population homozygous for the presence of *Fhb1* on their associated traits in the *Fhb1-* population homozygous for the absence of *Fhb1*. Traits values were averaged across 2011 and 2012 environments unless otherwise noted. Results were obtained via single marker analysis.

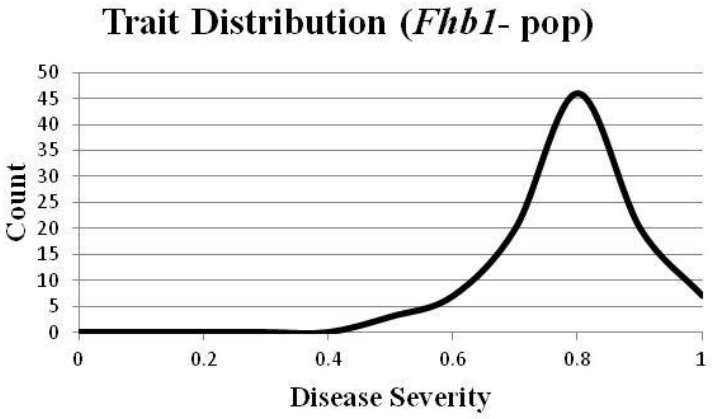
Trait	QTL	Position (cM)	P-value	R^2 (%)
FHB Incidence-2012-St. Paul	*Fhb2*	43.6	0.0020	11.1
DON Concentration-2012-St. Paul	*Fhb2*	43.6	0.0030	10.0
DON Concentration	*Fhb2*	43.6	0.0330	5.4
FHB Severity	*Fhb2*	43.6	0.0004	14.2
DON Concentration	*2A*	49.1	0.0030	9.6
FHB Severity	*2A*	49.1	0.0640	3.9

Figure 1: Schematic showing phenotypic results of F_3 family testing conducted by Liu *et al*. (2008) using the resistant near-isogenic line derived from the Sumai 3/Stoa//MN97448 population, '260-2,' and 'Bobwhite' as parents.

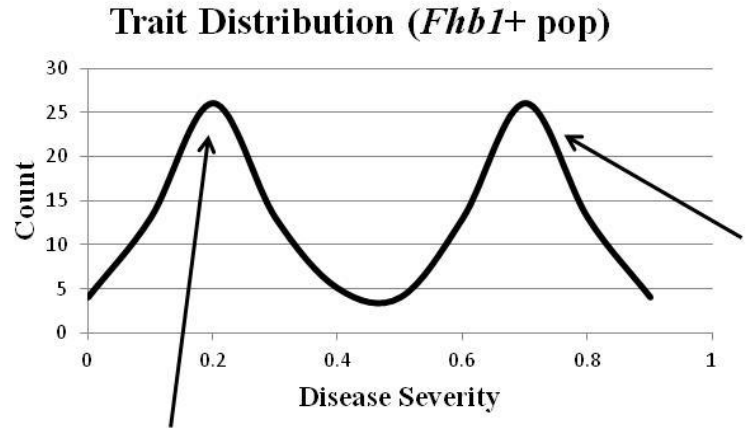


R = Resistant FHB spread phenotype in greenhouse testing
S = Susceptible FHB spread phenotype in greenhouse testing

Figure 2: Histograms displaying the phenotypic effect expected in each of two populations (*Fhb1*+ homozygous for presence of *Fhb1*, *Fhb1*- homozygous for absence of *Fhb1*) if a resistance gene suppressor is present (1 = most severe infection possible)



Effect of mapped QTL on all disease traits (more severe infection to right) in population lacking *Fhb1*.



Effect of *Fhb1* and mapped QTL on disease traits with presence of suppressor loci (more severe infection)

Effect of *Fhb1* and minor effect QTL on disease traits in absence of suppressor loci (high level of resistance)

Figure 3: Histograms of FHB resistance traits (Greenhouse Severity, FHB Severity, VSK, DON) and correlated traits (Heading Date, Plant Height, Micro Test Weight, 30 Head Weight) based on trait means across reps and locations for the *Fhb1+* and *Fhb1-* populations.

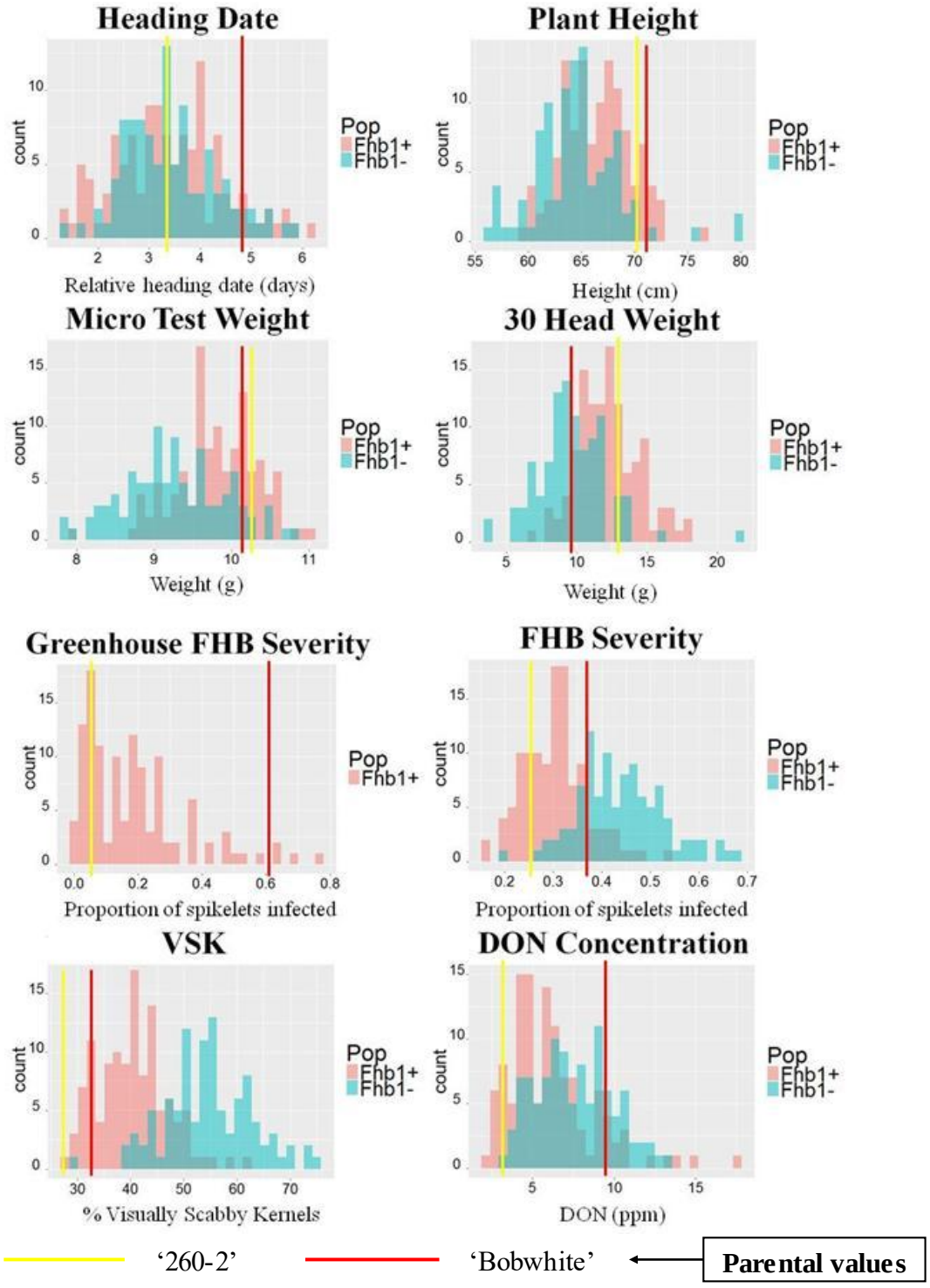
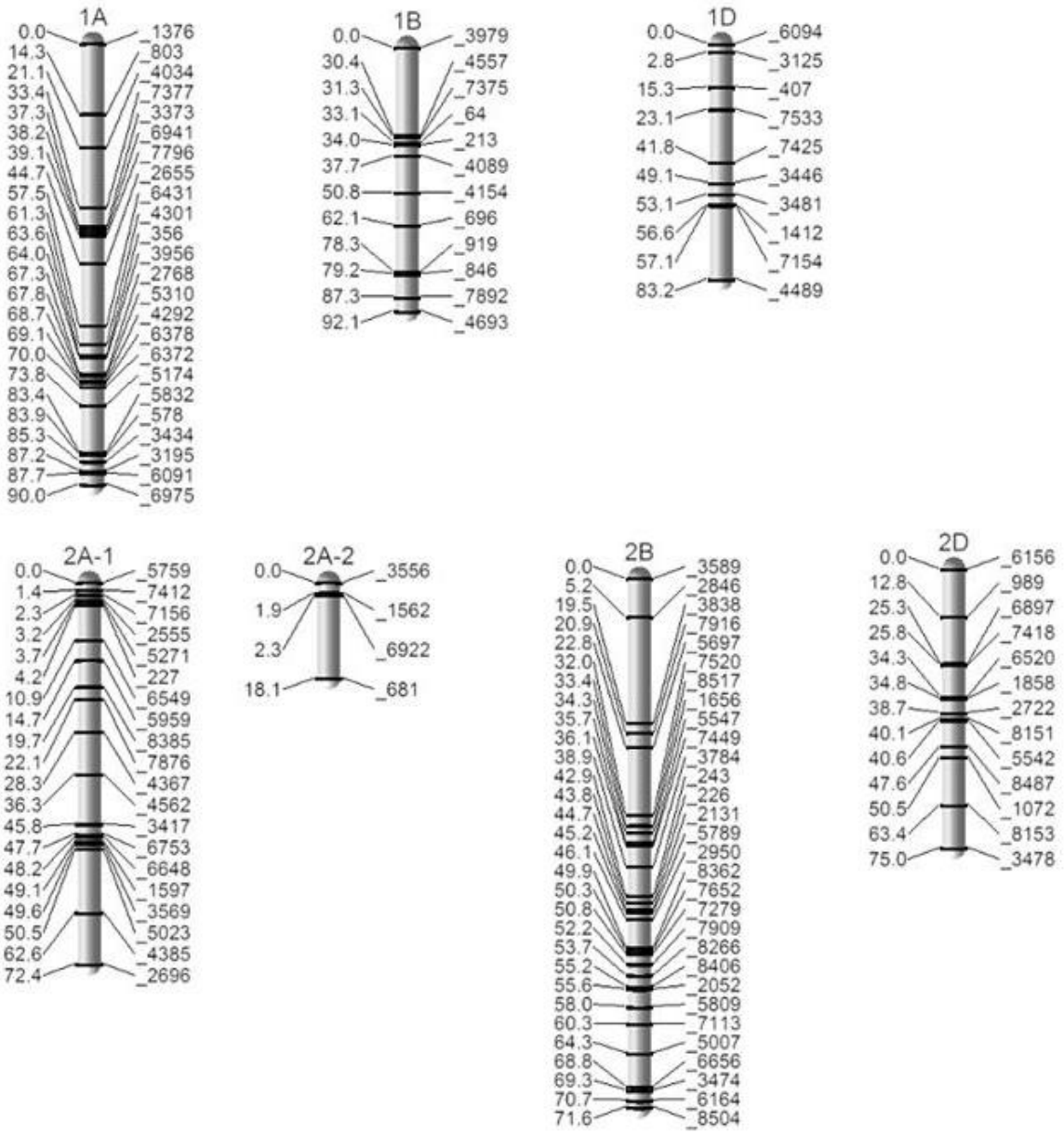
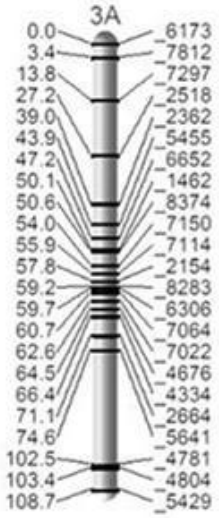
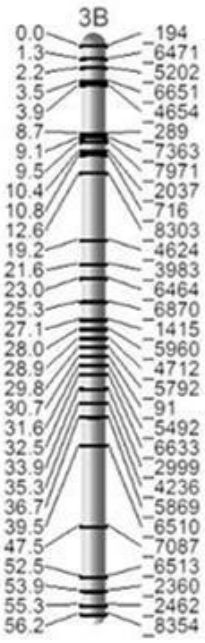


Figure 4: Linkage maps of the *Fhb1+* population (homozygous for presence of *Fhb1*) with SNP index numbers on the right and cM distance on the left. Chromosomal identities are shown above each linkage group.




3A
0.0 _6173
3.4 _7812
13.8 _7297
27.2 _2518
39.0 _2362
43.9 _5455
47.2 _6652
50.1 _1462
50.6 _8374
54.0 _7150
55.9 _7114
57.8 _2154
59.2 _8283
59.7 _6306
60.7 _7064
62.6 _7022
64.5 _4676
66.4 _4334
71.1 _2664
74.6 _5641
102.5 _4781
103.4 _4804
108.7 _5429


3B
0.0 _194
1.3 _6471
2.2 _5202
3.5 _6651
3.9 _4654
8.7 _289
9.1 _7363
9.5 _7971
10.4 _2037
10.8 _716
12.6 _8303
19.2 _4624
21.6 _3983
23.0 _6464
25.3 _6870
27.1 _1415
28.0 _5960
28.9 _4712
29.8 _5792
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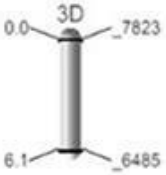
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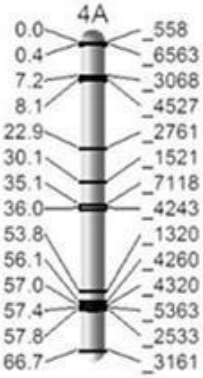
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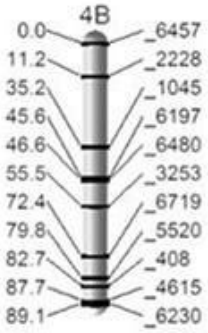
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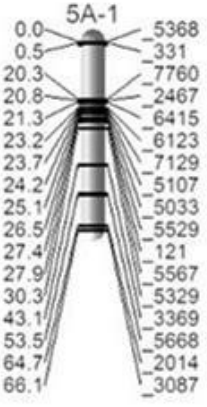
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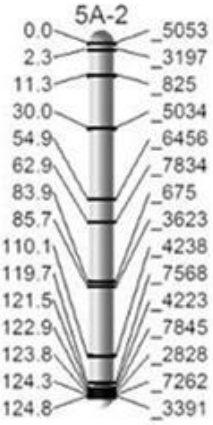
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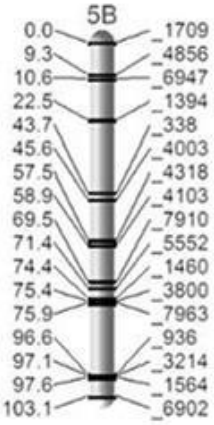
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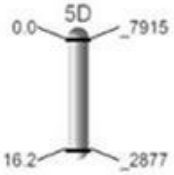
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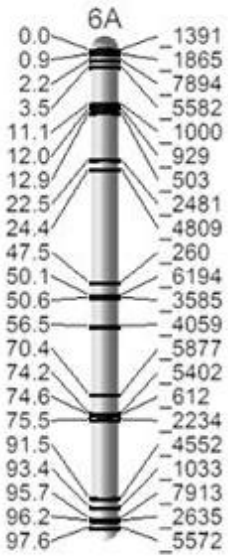
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47.5 _260
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50.6 _3585
56.5 _4059
70.4 _5877
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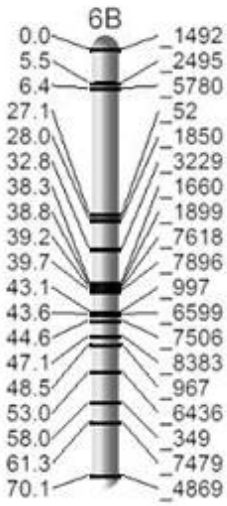
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43.1 _997
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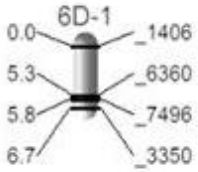
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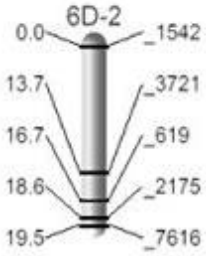
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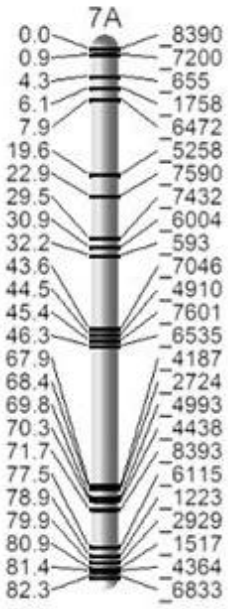
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46.3 _6535
67.9 _4187
68.4 _2724
69.8 _4993
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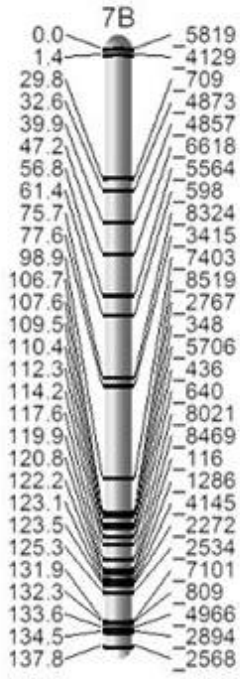
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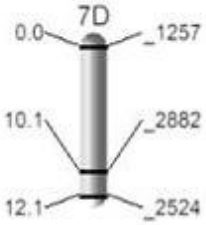
7D
0.0 _1257
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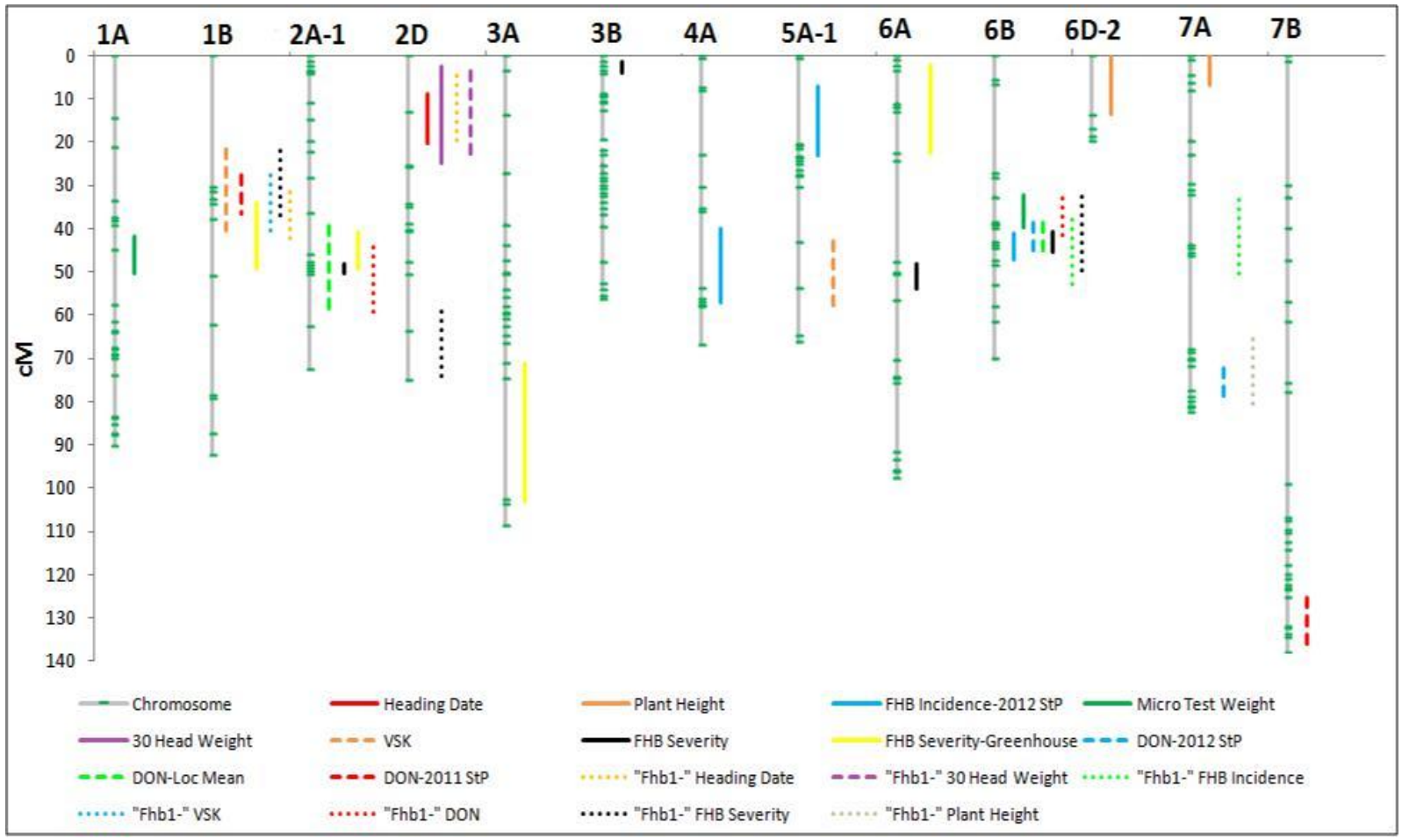
12.1 _2524

Figure 5: Summary of chromosomal QTL locations with LOD in excess of 3.2 in both *Fhb1+* (containing *Fhb1*) and *Fhb1-* (lacking *Fhb1*) populations. QTL intervals are shown to 2 standard deviations.



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Supplementary Table 1: Mean and standard deviation for *Fhb1+* population RILs across all environments and replications for FHB-related traits.

Genotype	Incidence		Severity		30 Head Weight		VSK		DON		Greenhouse	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
4-3	0.93	0.071	0.25	0.034	12.11	1.18	29.8	5.34	3.0	1.30	0.05	0.048
6-1	0.99	0.035	0.43	0.032	5.59	0.87	44.4	9.01	4.2	2.87	0.05	0.026
7-2	0.93	0.000	0.31	0.034	11.65	2.60	33.3	7.50	3.9	2.42	0.19	0.105
10-2	0.83	0.071	0.36	0.049	10.11	2.24	41.2	11.09	2.5	1.86	0.02	0.018
12-3	0.91	0.071	0.28	0.051	11.22	1.20	43.8	8.02	3.4	1.09	0.20	0.152
14-2	0.91	0.283	0.28	0.060	11.24	1.83	34.0	2.64	4.1	1.50	0.63	0.199
17-1	0.84	0.000	0.18	0.028	13.59	3.02	31.4	3.43	3.5	1.90	0.20	0.123
18-1	0.95	0.106	0.30	0.074	10.25	1.03	41.1	8.39	4.7	3.03	0.25	0.028
22-1	0.82	0.106	0.21	0.054	12.83	1.72	35.8	4.22	3.1	1.70	0.12	0.095
25-1	0.98	0.035	0.36	0.089	13.04	2.07	41.3	5.12	5.6	2.35	0.02	0.023
27-1	0.88	0.141	0.22	0.021	9.58	2.10	46.1	9.19	7.3	2.51	0.15	0.056
30-1	0.96	0.106	0.37	0.029	11.39	1.45	41.8	3.13	5.0	3.68	0.37	0.294
32-1	0.93	0.071	0.33	0.038	9.86	2.12	31.8	5.50	2.8	0.81	0.17	0.003
33-1	0.92	0.177	0.25	0.090	10.31	2.26	48.8	4.11	7.3	3.12	0.26	0.033
36-1	0.96	0.106	0.24	0.032	11.98	1.51	29.8	4.64	4.3	2.78	0.20	0.027
41-1	0.96	0.071	0.30	0.061	10.33	1.34	36.1	10.78	3.8	2.44	0.08	0.051
43-1	0.88	0.071	0.30	0.050	17.06	2.29	39.2	2.91	4.0	2.20	0.07	0.069
45-1	0.88	0.177	0.23	0.013	13.90	1.16	41.6	10.38	3.6	1.36	0.19	0.099
52-1	0.97	0.000	0.32	0.065	12.43	2.21	37.0	6.01	3.6	2.79	0.10	0.160
58-1	0.93	0.141	0.34	0.077	12.09	1.82	40.2	2.46	4.7	2.56	0.20	0.160
59-3	0.92	0.106	0.33	0.042	11.13	2.75	42.9	3.58	4.2	1.31	0.49	0.059
60-1	0.98	0.106	0.41	0.089	11.11	2.87	41.3	7.12	6.1	2.45	0.14	0.095
61-1	0.97	0.000	0.34	0.061	11.25	1.32	33.8	9.43	3.3	2.95	0.10	0.044
65-2	1.00	0.000	0.37	0.038	11.75	1.49	44.0	6.42	8.7	5.49	0.28	0.123
66-1	0.94	0.071	0.30	0.090	10.10	2.38	36.9	2.20	2.9	0.89	0.05	0.015
69-1	0.98	0.071	0.33	0.051	9.93	1.95	38.0	2.15	5.8	3.85	0.35	0.182
71-1	0.92	0.106	0.24	0.065	10.46	1.98	28.9	6.31	4.9	4.55	0.04	0.004
74-1	0.94	0.106	0.22	0.071	11.81	2.49	43.1	5.45	7.0	4.65	0.22	0.095
75-3	0.98	0.000	0.34	0.039	10.61	1.13	40.2	4.34	3.7	0.66	0.48	0.030
77-1	0.89	0.212	0.19	0.087	12.09	1.21	28.5	5.92	3.9	2.92	0.02	0.027
79-1	0.92	0.035	0.26	0.033	16.91	1.45	32.9	4.09	6.4	5.80	0.08	0.047
83-1	0.86	0.247	0.25	0.020	13.56	2.32	35.7	2.51	3.9	2.92	0.27	0.089
85-1	0.95	0.000	0.33	0.090	10.17	1.96	41.0	5.63	4.4	1.43	0.30	0.055
89-1	0.99	0.035	0.33	0.049	13.68	1.44	37.7	9.90	5.8	2.85	0.27	0.077
91-2	0.88	0.071	0.27	0.051	14.48	2.39	30.6	3.22	5.1	3.89	0.08	0.069
93-2	0.94	0.141	0.30	0.051	17.35	1.13	37.5	6.43	5.7	1.20	0.16	0.110
96-1	0.88	0.106	0.27	0.063	13.66	2.27	30.0	4.23	4.5	2.26	0.06	0.070
98-1	0.98	0.000	0.50	0.061	14.12	1.17	38.9	6.64	7.0	7.09	0.76	0.150
102-1	0.93	0.283	0.35	0.094	9.07	1.57	45.9	10.98	8.0	7.19	0.14	0.074
108-1	0.95	0.106	0.58	0.057	5.95	1.26	47.8	6.16	12.0	23.69	0.54	0.039
109-2	0.99	0.000	0.27	0.052	10.87	2.60	29.0	5.35	4.1	2.33	0.01	0.010
110-1	0.99	0.035	0.45	0.062	11.34	1.20	39.0	2.43	8.5	9.50	0.49	0.265
116-1	0.98	0.035	0.33	0.064	8.82	1.24	37.8	3.59	8.6	8.94	0.25	0.071
119-1	0.88	0.177	0.14	0.033	12.26	1.79	25.4	3.06	4.5	3.34	0.01	0.004

Genotype	Incidence		Severity		30 Head Weight		VSK		DON		Greenhouse	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
121-2	0.90	0.141	0.29	0.047	11.95	1.16	36.4	7.89	5.2	5.41	0.38	0.186
122-2	0.87	0.000	0.21	0.051	8.22	1.50	36.7	6.87	4.3	3.66	0.07	0.015
126-1	1.00	0.000	0.44	0.073	10.30	4.18	44.4	4.08	7.7	5.53	0.42	0.164
127-1	0.94	0.106	0.33	0.109	10.14	1.38	48.7	8.71	4.2	3.29	0.21	0.047
129-1	0.84	0.247	0.30	0.048	14.93	1.74	40.3	8.40	4.9	5.23	0.03	0.042
130-1	1.00	0.000	0.36	0.058	7.95	2.58	37.2	7.86	7.2	7.47	0.28	0.013
131-1	0.98	0.000	0.29	0.015	11.48	3.30	34.2	3.41	8.6	7.63	0.53	0.140
140-1	0.98	0.035	0.40	0.110	10.06	1.06	49.3	10.35	6.2	4.48	0.06	0.014
141-1	0.98	0.106	0.35	0.083	14.09	1.28	34.7	5.16	5.9	3.72	0.06	0.053
142-2	1.00	0.000	0.43	0.051	8.64	1.58	44.7	2.09	9.5	12.10	0.33	0.113
145-1	0.88	0.106	0.20	0.038	16.63	2.55	26.8	5.37	5.5	5.50	0.27	0.079
147-1	0.99	0.000	0.40	0.087	11.99	1.53	38.9	4.30	8.8	2.40	0.07	0.047
152-1	0.99	0.035	0.37	0.040	12.36	1.76	42.8	7.15	7.8	6.74	0.42	0.043
153-1	1.00	0.000	0.37	0.057	12.91	1.77	45.3	5.57	7.3	7.29	0.22	0.153
155-1	0.89	0.141	0.26	0.046	15.77	0.79	30.4	5.54	4.3	0.40	0.13	0.097
156-1	0.90	0.106	0.18	0.052	12.59	1.22	31.9	5.28	4.4	3.10	0.19	0.096
159-1	0.96	0.071	0.28	0.016	9.87	2.70	39.5	5.92	4.5	2.42	0.07	0.054
160-1	0.96	0.071	0.34	0.050	10.36	2.71	41.9	7.33	5.0	4.20	0.19	0.087
163-1	0.96	0.035	0.26	0.030	14.24	1.78	33.0	5.97	5.5	5.15	0.28	0.183
168-1	0.99	0.035	0.35	0.050	9.46	1.81	59.0	11.37	6.0	7.32	0.03	0.027
170-2	0.92	0.071	0.29	0.037	9.85	0.61	41.7	2.59	4.2	2.66	0.01	0.008
173-3	0.83	0.247	0.26	0.052	13.76	2.08	31.0	3.09	6.3	8.02	0.17	0.134
174-1	0.88	0.247	0.22	0.050	14.67	1.21	37.8	3.53	6.8	7.00	0.17	0.048
176-3	0.88	0.247	0.27	0.081	12.40	1.57	40.7	7.14	4.4	3.29	0.04	0.022
178-1	0.97	0.035	0.33	0.092	7.73	2.35	53.6	11.89	6.0	6.60	0.11	0.027
179-1	0.97	0.035	0.31	0.124	13.43	0.46	33.9	3.07	7.7	6.28	0.67	0.194
180-2	0.98	0.000	0.29	0.036	16.37	0.54	37.3	13.39	5.5	4.67	0.13	0.055
181-3	0.96	0.035	0.35	0.022	12.32	1.40	46.3	5.32	6.7	4.64	0.36	0.210
182-1	0.92	0.177	0.31	0.056	9.89	1.41	52.4	5.72	10.9	14.51	0.48	0.114
183-2	0.96	0.177	0.38	0.079	8.78	2.67	50.2	6.37	10.6	10.46	0.19	0.052
184-2	1.00	0.000	0.43	0.114	10.60	1.78	42.9	7.59	12.4	9.38	0.03	0.036
185-2	0.98	0.035	0.36	0.025	10.43	1.23	42.8	11.30	5.0	3.90	0.21	0.052
187-2	0.96	0.000	0.33	0.084	10.96	2.02	45.2	8.13	5.7	6.89	0.09	0.005
190-3	0.95	0.000	0.29	0.050	13.19	1.36	37.6	8.88	3.9	1.73	0.03	0.035
192-3	0.95	0.141	0.26	0.038	12.75	3.46	45.0	7.96	7.6	7.40	0.25	0.133
193-1	0.91	0.035	0.19	0.073	15.29	1.01	40.4	3.16	10.6	4.50	0.08	0.001
197-1	0.97	0.035	0.39	0.007	9.67	1.48	37.8	2.96	5.1	2.05	0.09	0.152
198-1	0.79	0.071	0.19	0.063	13.47	3.04	36.2	8.40	2.9	1.51	0.04	0.046
199-3	0.98	0.035	0.32	0.045	14.03	1.57	45.9	13.28	5.1	5.89	0.21	0.201
201-1	0.94	0.035	0.32	0.070	11.77	1.48	49.5	7.55	6.1	3.84	0.12	0.087
202-1	0.81	0.141	0.25	0.058	13.97	0.80	36.8	2.95	5.4	5.58	0.05	0.059
203-1	0.81	0.071	0.21	0.040	14.33	0.90	35.5	3.38	5.9	5.34	0.02	0.022
204-1	0.94	0.071	0.31	0.064	12.67	0.67	32.9	6.09	5.1	1.42	0.10	0.076
206-3	0.86	0.389	0.26	0.047	10.26	2.12	27.1	3.67	4.0	1.02	0.04	0.022
208-3	0.85	0.106	0.32	0.044	11.14	1.94	42.1	5.10	3.7	4.29	0.06	0.060
212-1	0.93	0.106	0.29	0.057	12.17	0.86	43.8	5.43	4.2	3.22	0.37	0.276
214-2	0.84	0.247	0.24	0.029	12.14	0.53	30.4	7.08	3.2	1.87	0.00	0.006

Genotype	Incidence		Severity		30 Head Weight		VSK		DON		Greenhouse	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
215-2	0.86	0.000	0.22	0.041	14.54	1.53	31.1	5.14	6.9	1.35	0.19	0.018
217-1	0.80	0.106	0.25	0.033	13.69	2.90	49.5	3.98	2.7	0.70	0.12	0.066
220-2	0.97	0.035	0.29	0.043	12.60	3.23	40.5	8.16	4.0	1.32	0.13	0.046
221-1	0.86	0.035	0.25	0.045	12.57	1.87	32.8	4.25	4.4	2.80	0.23	0.196
225-1	0.94	0.141	0.27	0.062	10.38	1.74	39.8	7.82	4.8	3.99	0.05	0.035
226-1	0.81	0.071	0.17	0.046	13.08	1.64	27.4	5.91	3.3	1.91	0.21	0.092
228-1	0.87	0.035	0.20	0.076	15.46	3.00	24.7	5.15	4.7	1.23	0.02	0.016
231-1	0.81	0.035	0.26	0.035	14.60	3.22	35.8	5.18	4.8	3.16	0.21	0.136
232-1	0.90	0.071	0.26	0.049	10.67	2.45	39.6	6.47	4.1	3.14	0.21	0.101
233-1	0.89	0.035	0.29	0.022	14.40	3.68	33.3	5.95	5.5	1.42	0.27	0.150
234-3	0.97	0.106	0.28	0.049	12.58	0.71	37.5	2.91	6.3	4.12	0.06	0.018
235-2	0.93	0.035	0.24	0.052	9.43	3.44	43.3	4.21	5.2	4.53	0.12	0.051
236-2	0.67	0.000	0.16	0.071	12.92	1.37	40.9	6.56	2.9	1.65	0.02	0.003
237-3	0.96	0.106	0.30	0.074	11.04	1.41	39.2	7.63	4.7	3.12	0.06	0.041
238-1	1.00	0.000	0.44	0.075	9.36	1.56	39.0	7.59	7.7	7.20	0.07	0.040
240-1	0.91	0.354	0.39	0.122	7.97	1.94	50.0	5.74	5.0	3.77	0.22	0.136
244-1	0.84	0.283	0.29	0.045	15.30	2.53	33.2	12.85	3.1	0.83	0.14	0.050
245-3	0.90	0.071	0.26	0.092	10.41	1.39	29.5	6.82	4.7	1.85	0.19	0.040
247-1	0.89	0.354	0.38	0.101	12.06	0.68	34.2	6.47	4.2	4.06	0.20	0.124
251-1	1.00	0.000	0.30	0.048	9.42	0.51	42.7	6.90	4.9	4.13	0.25	0.141
252-1	0.93	0.212	0.28	0.055	18.57	1.87	36.1	6.63	5.5	4.33	0.07	0.022
254-1	0.95	0.106	0.27	0.046	10.80	1.66	31.5	4.29	6.6	4.41	0.04	0.043
259-1	0.68	0.071	0.25	0.038	15.87	2.10	29.6	5.43	2.2	1.06	0.01	0.003
262-1	0.93	0.212	0.32	0.048	11.55	2.53	33.2	8.94	6.8	2.40	0.04	0.019
264-1	0.88	0.177	0.24	0.044	13.42	3.60	39.8	5.39	3.8	1.93	0.04	0.023
Parents and Checks												
260-2*	0.86	0.071	0.33	0.076	13.74	1.74	27.7	4.37	2.9	1.75	0.10	0.075
Bobwhite**	0.99	0.000	0.36	0.064	10.07	1.08	36.0	3.90	10.5	3.81	0.67	0.136
Alsen*	0.91	0.071	0.25	0.039	12.77	0.83	20.7	13.32	4.2	1.69	0.07	0.017
Wheaton***	1.00	0.000	0.62	0.169	7.29	3.20	70.7	9.62	7.9	4.48	0.69	0.047
260-4**	0.99	0.000	0.57	0.135	7.89	2.67	56.4	5.46	3.5	2.46	0.52	0.021
BacUp*	0.72	0.177	0.26	0.112	16.63	1.55	18.1	6.36	3.1	2.23		
MN00269***	1.00	0.000	0.62	0.138	4.71	1.56	47.9	5.20	9.0	6.15		
Roblin***	1.00	0.000	0.60	0.093	10.77	1.26	44.6	5.29	5.4	0.89		

***--Moderately resistant check

****--Moderately susceptible check

*****--Susceptible check

Supplementary Table 2: SNP names from the 9K Infinium chip corresponding to index numbers on linkage maps.

SNP Index #	SNP Name	SNP Index #	SNP Name
52	wsnp_BE404947B_Ta_2_12	989	wsnp_CAP12_c812_428290
64	wsnp_BE405834B_Ta_2_3	997	wsnp_CAP12_rep_c3885_1758057
91	wsnp_BE426222B_Ta_2_1	1000	wsnp_CAP12_rep_c4048_1842112
116	wsnp_BE443010B_Ta_2_1	1033	wsnp_CAP7_c1339_673581
121	wsnp_BE443187A_Ta_2_2	1045	wsnp_CAP7_c1723_854530
194	wsnp_BE446462D_Ta_2_1	1072	wsnp_CAP7_c2782_1329707
213	wsnp_BE489692B_Ta_2_2	1223	wsnp_CAP8_rep_c3844_1896355
226	wsnp_BE490267A_Ta_2_1	1257	wsnp_cd454041D_Ta_2_1
227	wsnp_BE490384A_Ta_2_1	1286	wsnp_Ex_c10124_16630607
237	wsnp_BE490744A_Td_2_1	1320	wsnp_Ex_c10390_17007929
243	wsnp_BE490763B_Ta_2_2	1376	wsnp_Ex_c10657_17376448
260	wsnp_BE495143A_Ta_2_2	1391	wsnp_Ex_c10824_17611901
289	wsnp_BE497169B_Ta_2_2	1394	wsnp_Ex_c10842_17637744
331	wsnp_BE499835A_Ta_2_2	1406	wsnp_Ex_c10910_17729008
338	wsnp_BE517711B_Ta_2_2	1412	wsnp_Ex_c10959_17801482
348	wsnp_BE518357B_Ta_2_2	1415	wsnp_Ex_c1097_2104146
349	wsnp_BE518379B_Ta_2_2	1460	wsnp_Ex_c11265_18216936
356	wsnp_BE585780A_Ta_2_1	1462	wsnp_Ex_c11297_18254062
407	wsnp_BE637971D_Ta_2_3	1492	wsnp_Ex_c1143_2194680
408	wsnp_BE638137B_Ta_2_2	1517	wsnp_Ex_c1159_2224684
436	wsnp_BF291608B_Ta_2_1	1521	wsnp_Ex_c11619_18714738
503	wsnp_BF483091A_Ta_2_2	1542	wsnp_Ex_c1184_2272501
558	wsnp_BG313770B_Ta_1_1	1562	wsnp_Ex_c11950_19164041
578	wsnp_BG606986A_Ta_2_4	1564	wsnp_Ex_c11951_19164786
593	wsnp_BM134363A_Ta_2_4	1597	wsnp_Ex_c12219_19526749
598	wsnp_BM137749D_Ta_2_1	1656	wsnp_Ex_c12572_20015503
612	wsnp_BQ159493A_Ta_2_2	1660	wsnp_Ex_c12618_20079758
619	wsnp_BQ161779D_Ta_2_1	1709	wsnp_Ex_c12927_20480163
640	wsnp_CAP11_c103_134545	1758	wsnp_Ex_c13337_21022241
655	wsnp_CAP11_c1182_686503	1850	wsnp_Ex_c14031_21934322
675	wsnp_CAP11_c1506_840951	1858	wsnp_Ex_c14107_22021215
681	wsnp_CAP11_c1604_886894	1865	wsnp_Ex_c14156_22088409
696	wsnp_CAP11_c1902_1022590	1899	wsnp_Ex_c14473_22473602
709	wsnp_CAP11_c2255_1176515	2014	wsnp_Ex_c15342_23592789
716	wsnp_CAP11_c232_212121	2037	wsnp_Ex_c1558_2976128
803	wsnp_CAP11_c710_458019	2052	wsnp_Ex_c15681_24015996
809	wsnp_CAP11_c802_502739	2131	wsnp_Ex_c16425_24923837
825	wsnp_CAP11_c951_572693	2154	wsnp_Ex_c1660_3159173
846	wsnp_CAP11_rep_c4138_1957291	2175	wsnp_Ex_c1690_3206784
919	wsnp_CAP12_c1337_682282	2228	wsnp_Ex_c17561_26284693
929	wsnp_CAP12_c1663_836928	2234	wsnp_Ex_c17575_26300030
936	wsnp_CAP12_c2231_1090724	2272	wsnp_Ex_c1790_3378771
967	wsnp_CAP12_c475_258416	2360	wsnp_Ex_c18915_27811736

SNP Index #	SNP Name
2362	wsnp_Ex_c1894_3575749
2462	wsnp_Ex_c19778_28779907
2467	wsnp_Ex_c19820_28829623
2481	wsnp_Ex_c19928_28951983
2495	wsnp_Ex_c20_42503
2518	wsnp_Ex_c20250_29303152
2524	wsnp_Ex_c20320_29384395
2533	wsnp_Ex_c20386_29451037
2534	wsnp_Ex_c204_400545
2555	wsnp_Ex_c20771_29858497
2568	wsnp_Ex_c2103_3947695
2635	wsnp_Ex_c21633_30782312
2655	wsnp_Ex_c2181_4089639
2664	wsnp_Ex_c21950_31124594
2696	wsnp_Ex_c22202_31392780
2722	wsnp_Ex_c2251_4218338
2724	wsnp_Ex_c22547_31738007
2761	wsnp_Ex_c2288_4293430
2767	wsnp_Ex_c22955_32173776
2768	wsnp_Ex_c22963_32183009
2828	wsnp_Ex_c23689_32927141
2846	wsnp_Ex_c2388_4476302
2877	wsnp_Ex_c24145_33394561
2882	wsnp_Ex_c24179_33428275
2894	wsnp_Ex_c24376_33619527
2898	wsnp_Ex_c24432_33676448
2929	wsnp_Ex_c24796_34049469
2950	wsnp_Ex_c2510_4689918
2999	wsnp_Ex_c2580_4799370
3068	wsnp_Ex_c26776_36003586
3087	wsnp_Ex_c27046_36265198
3125	wsnp_Ex_c278_538285
3161	wsnp_Ex_c28429_37553452
3195	wsnp_Ex_c28900_37982485
3197	wsnp_Ex_c28908_37989320
3214	wsnp_Ex_c29130_38196906
3229	wsnp_Ex_c2936_5416717
3253	wsnp_Ex_c29867_38850724
3350	wsnp_Ex_c31584_40355363
3369	wsnp_Ex_c31914_40647363
3373	wsnp_Ex_c31983_40709607
3391	wsnp_Ex_c32414_41076471
3415	wsnp_Ex_c32905_41484291
3417	wsnp_Ex_c32910_41489631
3434	wsnp_Ex_c33452_41938013
3446	wsnp_Ex_c3372_6195001
3474	wsnp_Ex_c34303_42642389

SNP Index #	SNP Name
3478	wsnp_Ex_c34419_42734849
3481	wsnp_Ex_c3450_6323939
3556	wsnp_Ex_c36049_44083089
3569	wsnp_Ex_c36242_44232305
3585	wsnp_Ex_c36801_44683992
3589	wsnp_Ex_c3685_6723631
3623	wsnp_Ex_c3772_6866645
3721	wsnp_Ex_c40522_47561668
3784	wsnp_Ex_c41558_48355943
3800	wsnp_Ex_c4189_7565086
3838	wsnp_Ex_c4272_7708423
3956	wsnp_Ex_c4605_8240189
3979	wsnp_Ex_c4668_8353466
3983	wsnp_Ex_c47078_52393295
4003	wsnp_Ex_c47991_53037623
4034	wsnp_Ex_c4876_8692849
4059	wsnp_Ex_c4954_8813973
4089	wsnp_Ex_c5098_9047611
4103	wsnp_Ex_c5155_9140608
4129	wsnp_Ex_c52259_55922750
4145	wsnp_Ex_c52527_56097039
4154	wsnp_Ex_c5296_9365847
4187	wsnp_Ex_c53442_56678505
4223	wsnp_Ex_c54211_57168122
4236	wsnp_Ex_c5457_9632050
4238	wsnp_Ex_c54655_57455110
4243	wsnp_Ex_c5470_9657856
4260	wsnp_Ex_c55245_57821389
4292	wsnp_Ex_c56097_58352130
4301	wsnp_Ex_c5634_9906981
4318	wsnp_Ex_c56866_58816359
4320	wsnp_Ex_c5690_9994305
4334	wsnp_Ex_c57322_59084809
4364	wsnp_Ex_c5839_10246915
4367	wsnp_Ex_c5856_10276064
4385	wsnp_Ex_c59373_60260876
4438	wsnp_Ex_c61603_61581245
4489	wsnp_Ex_c6378_11087794
4527	wsnp_Ex_c6514_11307200
4552	wsnp_Ex_c6604_11441822
4557	wsnp_Ex_c6611_11452297
4562	wsnp_Ex_c6660_11526924
4615	wsnp_Ex_c7059_12151409
4624	wsnp_Ex_c7108_12222660
4654	wsnp_Ex_c7316_12552186
4676	wsnp_Ex_c742_1458743
4693	wsnp_Ex_c750_1474300

SNP Index #	SNP Name
4712	wsnp_Ex_c76693_73578311
4781	wsnp_Ex_c8240_13914674
4804	wsnp_Ex_c8409_14170476
4809	wsnp_Ex_c8510_14306239
4856	wsnp_Ex_c8962_14947544
4857	wsnp_Ex_c8963_14948293
4869	wsnp_Ex_c9038_15058444
4873	wsnp_Ex_c908_1754208
4910	wsnp_Ex_c9428_15641609
4926	wsnp_Ex_c9483_15722127
4966	wsnp_Ex_c9813_16193536
4993	wsnp_Ex_c9971_16412615
5007	wsnp_Ex_rep_c101342_86720058
5023	wsnp_Ex_rep_c101526_86881619
5033	wsnp_Ex_rep_c101757_87065032
5034	wsnp_Ex_rep_c101757_87065169
5053	wsnp_Ex_rep_c102143_87374435
5107	wsnp_Ex_rep_c103972_88799335
5174	wsnp_Ex_rep_c109742_92411838
5202	wsnp_Ex_rep_c66331_64502558
5258	wsnp_Ex_rep_c66540_64820281
5271	wsnp_Ex_rep_c66606_64905694
5310	wsnp_Ex_rep_c66846_65240088
5329	wsnp_Ex_rep_c66900_65314206
5363	wsnp_Ex_rep_c67145_65628860
5368	wsnp_Ex_rep_c67179_65674582
5402	wsnp_Ex_rep_c67468_66069282
5429	wsnp_Ex_rep_c67635_66292308
5455	wsnp_Ex_rep_c67786_66472568
5492	wsnp_Ex_rep_c68066_66815745
5520	wsnp_Ex_rep_c68248_67035459
5529	wsnp_Ex_rep_c68441_67261799
5542	wsnp_Ex_rep_c68555_67394261
5547	wsnp_Ex_rep_c68587_67434960
5552	wsnp_Ex_rep_c68600_67449494
5564	wsnp_Ex_rep_c68762_67626384
5567	wsnp_Ex_rep_c68829_67704044
5572	wsnp_Ex_rep_c68915_67808523
5582	wsnp_Ex_rep_c69054_67959458
5641	wsnp_Ex_rep_c69816_68774416
5668	wsnp_Ex_rep_c70117_69067356
5697	wsnp_Ex_rep_c70571_69488416
5706	wsnp_Ex_rep_c70700_69597262
5759	wsnp_Ex_rep_c90786_81061397
5780	wsnp_JD_c11061_11602784
5789	wsnp_JD_c11975_12326445
5792	wsnp_JD_c12087_12411036

SNP Index #	SNP Name
5809	wsnp_JD_c12346_12606967
5819	wsnp_JD_c1285_1848292
5832	wsnp_JD_c13384_13393159
5869	wsnp_JD_c15974_15272598
5877	wsnp_JD_c1683_2351689
5959	wsnp_JD_c2578_3489735
5960	wsnp_JD_c2623_3541255
6004	wsnp_JD_c3225_4227048
6091	wsnp_JD_c5144_6266384
6094	wsnp_JD_c5316_6447231
6115	wsnp_JD_c5861_7018974
6123	wsnp_JD_c6047_7211052
6156	wsnp_JD_c69_109951
6164	wsnp_JD_c7305_8404286
6173	wsnp_JD_c7528_8610786
6194	wsnp_JD_c8334_9342604
6197	wsnp_JD_c8515_9498794
6230	wsnp_JD_c9484_10319946
6306	wsnp_JD_rep_c64325_41024646
6360	wsnp_JG_c5646_2148296
6372	wsnp_Ku_c10065_16730445
6378	wsnp_Ku_c10239_16988077
6415	wsnp_Ku_c11110_18216209
6431	wsnp_Ku_c11769_19153951
6436	wsnp_Ku_c11846_19263340
6456	wsnp_Ku_c12211_19780409
6457	wsnp_Ku_c12399_20037334
6464	wsnp_Ku_c12544_20235135
6471	wsnp_Ku_c12698_20441325
6472	wsnp_Ku_c12701_20446223
6480	wsnp_Ku_c13052_20918857
6485	wsnp_Ku_c13204_21105694
6510	wsnp_Ku_c1391_2771050
6513	wsnp_Ku_c14082_22272647
6520	wsnp_Ku_c14251_22503965
6535	wsnp_Ku_c14678_23061894
6549	wsnp_Ku_c15077_23576192
6563	wsnp_Ku_c15531_24168235
6599	wsnp_Ku_c16522_25425455
6618	wsnp_Ku_c17161_26193672
6633	wsnp_Ku_c17718_26860963
6648	wsnp_Ku_c18282_27558405
6651	wsnp_Ku_c18473_27773912
6652	wsnp_Ku_c18497_27803432
6656	wsnp_Ku_c18587_27915541
6719	wsnp_Ku_c21787_31570491
6753	wsnp_Ku_c2413_4626451

SNP Index #	SNP Name
6833	wsnp_Ku_c28104_38042857
6870	wsnp_Ku_c30078_39930134
6897	wsnp_Ku_c3107_5818628
6902	wsnp_Ku_c3151_5892200
6922	wsnp_Ku_c33374_42877546
6941	wsnp_Ku_c34659_43981982
6947	wsnp_Ku_c35090_44349517
6975	wsnp_Ku_c3804_6986527
7022	wsnp_Ku_c40218_48484410
7046	wsnp_Ku_c42539_50247426
7064	wsnp_Ku_c44089_51445136
7087	wsnp_Ku_c458_954940
7101	wsnp_Ku_c46846_53471053
7113	wsnp_Ku_c48694_54811423
7114	wsnp_Ku_c4886_8753646
7118	wsnp_Ku_c4924_8816643
7129	wsnp_Ku_c5071_9049540
7150	wsnp_Ku_c5243_9344536
7154	wsnp_Ku_c53270_57959459
7156	wsnp_Ku_c53501_58106782
7200	wsnp_Ku_c5938_10491100
7262	wsnp_Ku_c7078_12236807
7279	wsnp_Ku_c7297_12596001
7297	wsnp_Ku_c7811_13387117
7363	wsnp_Ku_c9596_16057771
7375	wsnp_Ku_c9971_16598986
7377	wsnp_Ku_rep_c101175_88380491
7403	wsnp_Ku_rep_c103690_90365438
7412	wsnp_Ku_rep_c104451_90920763
7418	wsnp_Ku_rep_c105822_91859983
7425	wsnp_Ku_rep_c109593_94159536
7432	wsnp_Ku_rep_c113718_96236830
7449	wsnp_Ku_rep_c68888_68067293
7479	wsnp_Ku_rep_c70721_70356213
7496	wsnp_Ku_rep_c71567_71302046
7506	wsnp_Ku_rep_c72013_71735741
7520	wsnp_Ku_rep_c73313_72887199
7533	wsnp_Ra_c1020_2062200
7552	wsnp_Ra_c10911_17834481
7568	wsnp_Ra_c11532_18688426
7590	wsnp_Ra_c12708_20281439
7601	wsnp_Ra_c1308_2604804
7616	wsnp_Ra_c13881_21836489
7618	wsnp_Ra_c13949_21928888
7652	wsnp_Ra_c16333_24961476
7760	wsnp_Ra_c22491_31958067
7796	wsnp_Ra_c26191_35761997

SNP Index #	SNP Name
7812	wsnp_Ra_c27517_37034553
7823	wsnp_Ra_c28858_38278180
7834	wsnp_Ra_c29586_38941694
7845	wsnp_Ra_c3095_5835193
7876	wsnp_Ra_c3378_6318431
7892	wsnp_Ra_c35998_44436619
7894	wsnp_Ra_c36410_44760538
7896	wsnp_Ra_c3766_6947263
7909	wsnp_Ra_c3955_7262354
7910	wsnp_Ra_c39562_47242455
7913	wsnp_Ra_c3996_7334169
7915	wsnp_Ra_c40111_47657589
7916	wsnp_Ra_c407_862316
7963	wsnp_Ra_c47696_53184502
7971	wsnp_Ra_c488_1027573
8021	wsnp_Ra_c60161_61164295
8151	wsnp_Ra_rep_c116793_96612614
8153	wsnp_Ra_rep_c69210_66556925
8266	wsnp_RFL_Contig1892_1042675
8283	wsnp_RFL_Contig2011_1216801
8303	wsnp_RFL_Contig2177_1500201
8324	wsnp_RFL_Contig2315_1788036
8354	wsnp_RFL_Contig2569_2199100
8362	wsnp_RFL_Contig2612_2274356
8374	wsnp_RFL_Contig2699_2402527
8383	wsnp_RFL_Contig2747_2479869
8385	wsnp_RFL_Contig2763_2509104
8390	wsnp_RFL_Contig2789_2553657
8393	wsnp_RFL_Contig2805_2579582
8406	wsnp_RFL_Contig2914_2757372
8469	wsnp_RFL_Contig3405_3533915
8487	wsnp_RFL_Contig3561_3746066
8504	wsnp_RFL_Contig3712_3953814
8517	wsnp_RFL_Contig3802_4108582
8519	wsnp_RFL_Contig3817_4141066

Supplementary Table 3: Randomly selected and genotyped subset of the *Fhb1*+ RIL population comparing marker genotypes at the 2A QTL and microsatellite GWM382 linked to the previously mapped 'Stoa' QTL, *Qfhb.ndsu-2AL* (Waldron *et al*. 1999, Anderson *et al*. 2001). '260-2' indicates the resistant allele and 'Bobwhite' indicates the susceptible allele.

Genotype	GWM382	2A QTL
6-1	260-2	260-2
10-2	260-2	260-2
33-1	260-2	Bobwhite
59-3	260-2	Bobwhite
60-1	260-2	Bobwhite
83-1	Bobwhite	260-2
109-2	260-2	260-2
122-2	Bobwhite	260-2
140-1	Bobwhite	Bobwhite
142-2	Bobwhite	Bobwhite
176-3	260-2	260-2
178-1	Bobwhite	Bobwhite
182-1	Bobwhite	Bobwhite
187-2	260-2	260-2
204-1	260-2	Bobwhite
208-3	260-2	Bobwhite
221-1	260-2	Bobwhite
238-1	260-2	Bobwhite
245-3	Bobwhite	Bobwhite